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THE MECHANISM OF THE VICIOUS CIRCLE IN CHRONIC HYPERTENSION *

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IN the course of work curtailed by the outbreak of war, Wilson and Byrom (15, 16), found that in the rat, permanent hypertension could be caused by applying a simplified Goldblatt clamp to the artery of one kidney (the *ischæmic* kidney), that the hypertension was commonly followed by the appearance in the opposite (or *intact*) kidney of destructive vascular lesions, and that when this destruction had become sufficiently extensive, the hypertension could no longer be abolished by excising the ischæmic kidney Wilson and Byrom concluded that a vicious circle had been set in train and drew far-reaching inferences from this conclusion

The validity of the conception of the vicious circle rests on two deductions from the experimental results , firstly, that the damage in the intact kidney was caused by the original hypertension , and, secondly, that the residual hypertension was maintained by this damage   We have already reviewed the first deduction in the light of subsequent evidence (3), and our present object is to submit further data bearing on the second

Wilson and Byrom demonstrated a correlation between the extent of the damage in the intact kidney and the occurrence of residual hypertension   but recognised that this did not constitute proof that the two were cause and effect, and they admitted that an un-known factor, independent of the kidney, might have intruded to perpetuate the hypertension   The possibility of such an intrusion has long been suspected   for reasons which will now be outlined In the first place, it has been widely believed in the past that the generalised increase in arteriolar tone which is the essential abnormality in hypertension leads in time to an irreversible structural narrowing of the arterioles   This belief is probably erroneous, because although individual arteries may become narrowed by atheroma, the arteriolar tone in general can be relaxed by vasodilator mechanisms in the hypertensive as in the normal subject (8 12)   Apart from this possibility there is a growing suspicion that the humoral (renin) mechanism which initiates experimental renal hypertension is later superseded by a different mechanism, possibly neurogenic in

aiactei   This suspicion is supported by the following evidence   The
 nin content of the rabbit's kidney is increased in recent hypertension,
it not in chronic experiments (10) ,   moreover the response to injected
nin also increases as hypertension persists (9)   Experimental hypertension
not reduced when the sensitivity to renin has been abolished by massive
jections (14)   In the rat, Reed, Sapirstein, Southard and Ogden (13),
port that chronic, but not acute, hypertension is abolished by nembutal
iæsthesia and by the alkaloid yohimbine   They infer that a neurogenic
echanism replaces the renin-hypertensin system in chronic hypertension
ore direct evidence has been described by Pickering (9), working on rabbits
.th hypertension deriving from a solitary ischæmic kidney   He found that
ute hypertension (up to eight days), was promptly relieved by nephrectomy,
it that in chronic hypertension (7 to 15 weeks), the high pressure persisted
ter nephrectomy until the animal died in uræmia, three days later
.ckering concludes that in chronic experimental hypertension, " a new
in-renal factor plays an important and perhaps the chief role in maintaining
.e raised pressure "

Apart from this last experiment, which remains unexplained, the above
idence suggests that in some way the mechanism of renal hypertension
anges with time   To provide an alternative explanation of persistent
ypertension, however it is necessary to go further and to show that the new
echanism is no longer dependent on the persistence of renal ischæmia
'ilson and Byrom (unpublished experiments), attempted to study the
iestion in their rats, but the presence of two kidneys, the one ischæmic
id usually atrophied, the other affected to an uncertain extent by secondary
ypertensive damage, provided serious obstacles   In animals other than
.e rat, restriction of the blood supply to one of two kidneys usually causes
ily transient hypertension and the question of persistent hypertension
ies not, therefore, arise, but in one of Goldblatt's dogs (7) which was
eated in this way chronic hypertension followed and was abolished, ten
onths later, by excising the ischæmic kidney

In approaching the problem we have followed a slightly different path
' an independent extra-renal factor intrudes in the hypertensive rat with
ie ischæmic and one intact kidney, it is reasonable to expect a similar
.trusion in an animal with chronic hypertension caused by constricting one
nal artery after excising the opposite kidney   In such experiments it is
inerally agreed that the clamp on the renal artery protects the kidney
om hypertensive damage and the problem could be examined, and perhaps
icisively answered, by simply removing the clamp from the artery after
ypertension had persisted for an adequate period   If this procedure
igularly abolished the hypertension an independent extra-renal mechanism
iuld be excluded   Persistence of the hypertension, on the other hand,
ould be less easy to interpret, because it would be difficult to exclude

narrowing of the renal artery due to peri-arterial fibrosis at the site of the clamp and also permanent ischæmic damage in the kidney itself Perhaps for these reasons or because of technical difficulties this simple experiment seems to have been neglected for the only reports which we have been able to trace relate to comparatively acute experiments in the dog (1 4 6 7)

In a few preliminary experiments we have examined the question in the rabbit and have found that hypertension of from 6 to 8 weeks duration is abolished by removing the clamp the blood pressure returning to normal within 24 hours Most of our data however has been derived from the rat

Experimental Methods

The animals used in these experiments were young adult albino rats of both sexes weighing from 120 to 270 grammes The right kidney was excised and two weeks later a clip made from annealed silver tape 1 7 mm broad by 0 14 mm thick with a gap of 0 25 mm was placed on the left renal artery under ether anæsthesia The systolic blood pressure was measured on the tail by an oscilloscopic technique (2), using ether anæsthesia the anæsthetising box being warmed to 38° C Measurements were taken on at least two occasions before the clip was applied and thereafter at weekly intervals After a suitable period varying from 4½ to 32 weeks the clip was removed from the artery The blood pressure was measured frequently during the next few days and then weekly for at least four weeks The animal was then killed the carcase and heart were weighed and sections were taken from the kidney pancreas heart and occasionally from other organs, for histological study

RESULTS

The effect of clamping the renal artery

Clips were applied in 84 rats, of which 14 died within seven days, presumably from renal failure Of the survivors seven failed to develop hypertension within four weeks and were discarded The remaining 63 developed sustained hypertension The hypertension varied in degree but was in general more marked and less intermittent than that observed in rats with a second kidney and tended to increase as time went on As was expected, the incidence of complications was also much higher These complications resembled the " vascular crises " observed by Wilson and Byrom and others, and were usually preceded by an upward trend in the pressure curve Two main types, cardiac and cerebral were found The former was accompanied by weakness, thirst and polyuria, collapse, pallor, dyspnœa tachycardia and a low blood pressure, death occurring in from 2 to 48 hours Cardiac infarcts, associated with necrotic lesions of the coronary arteries, were usually found *post mortem*

The cerebral type of crisis was accompanied by severe tonic and clonic, generalised or local convulsions, and post-mortem examination sometimes revealed either a gross cerebral hæmorrhage or recent vascular necroses Other complications included arterial hæmorrhage into the eye, the bladder, the scrotum, the intestine and the peritoneum, all attributable to arterial necrosis   The mortality from these complications was very high and 27 rats died before the clamp could be removed   These formed a useful group for comparing the pathological changes with those described subsequently   In all except four rats necrotising arteritis was found in the heart, pancreas, or brain   The heart was invariably hypertrophied   The kidney was examined in 23 rats   It invariably appeared normal to the naked eye and in 16 kidneys no microscopical abnormality was detected Seven kidneys, however, showed minor changes, of which two varieties were noticed   The first consisted of narrow radial strands of simple atrophy, apparently ischæmic in nature   The second consisted of small collections of plasma cells, eosinophils and lymphocytes in the adventitia of large and small vessels, possibly a low grade inflammatory reaction spreading inwards from the clip   Both lesions were usually inconspicuous   Arterial necrosis was absent except in one kidney, which displayed a single recent arteriolar necrosis   The substantially normal appearance of the kidney confirms earlier reports on other species and contrasts sharply with the atrophy of the ischæmic kidney which is usually found in rats with a second intact
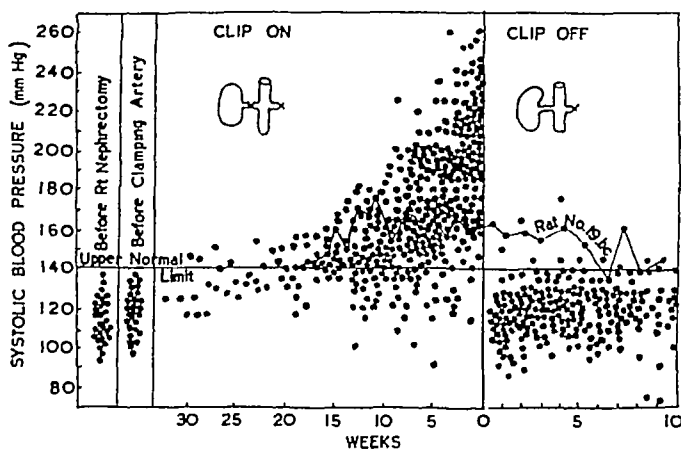
Fig 1   Systolic blood pressure readings from 34 rats with chronic hypertension produced by removing one kidney and constricting the artery to the other   Showing the effect of releasing the constriction

kidney   It is evident that when the whole renal territory is threatened with ischæmia, and providing this is not severe enough to cause acute renal insufficiency, a much more vigorous attempt is made to meet the challenge by raising the blood pressure, regardless of the disastrous consequences to other organs, such as the heart and brain

The above complications considerably reduced the experimental material and left only 34 rats available for removal of the clip   Twenty-three of these had symptoms of vascular crises at the time of this operation and would probably have died had the clip not been removed   The upward trend in the blood pressure readings for the 34 rats is well shown in Fig 1

The effect of removing the clamp

Blood pressure   With the exception of rat 19 BC, which will be separately considered, removal of the clip promptly and permanently abolished the hypertension, the blood pressure returning to the normal range within 24 hours   The full results are collected in Table I and illustrated in Fig 1

TABLE I

Systolic blood pressures before during and after constriction of the artery to a solitary kidney in the rat

Rat No	Before applying clip	Systolic blood pressure (mm Hg)					
		After applying clip			After removing clip		
		No of weekly readings	Range	Mean	No of weekly readings	Range	Mean
11 LC	— —	18	135–210	172	10	75–138	112
14 BC	135	11	132–230	180	10	110–146	125
15 BO	125, 120	11	100–220	170	5	110–120	115
15 LS	125, 130	7	125–215	158	11	120– 90	110
16 RC	133, 122	7	80–195	136	6	90–117	105
18 RC	122, 120	6	110–200	149	5	105–145	118
19 BO	112, 110	12	135–235	193	19	115–136	126
19 RC	105, 120	12	125–235	186	6	70–124	105
19 BC	114, 130	19	120–175	157	10	135–163	144
19 RS	112, 125	17	120–155	138	12	105–135	120
20 BO	95 90	20	115–235	172	8	110–128	121
20 RS	105 122	5	120–175	150	18	75–118	101
21 BO	125 120	8	120–215	163	16	105–135	124
21 LC	95 118	8	128–215	174	15	90–135	109
21 BC	102, 115	7	135–205	175	13	105–160	118
21 RS	100, 110	4	170–260	199	15	112–175	127
22 BO	110 105	5	148–245	193	16	120–140	127
22 BC	100, 105	13	118–250	182	7	115–150	131
22 LS	95, 110	30	125–210	154	5	100–135	116
23 BO	127, 116	11	172–255	206	7	135–110	126
23 RC	118, 122	19	135–240	172	7	112–140	123
23 BC	106, 125	13	150–225	196	7	115–130	123
24 RC	110, 112	15	135–245	194	7	130– 95	118
24 BC	108 —	9	152–240	200	9	105–125	117
26 RC	122, 112	32	120–205	151	5	105–140	127
26 LC	135, 102	20	135–195	165	6	122– 85	105
26 BC	117, 115	32	115–165	135	5	105–120	110
26 RS	105, 102	12	110–225	163	8	90–130	115
27 BO	108, 110	18	135–170	154	6	70–108	94
27 RC	104 98	13	130–220	173	7	103–138	121
27 LS	112, 135	13	140–260	190	7	107–130	122
28 LC	115, 110	9	100–205	169	8	122–135	129
29 RC	102, 135	20	125–240	188	8	125–145	130
29 RS	105, 100	13	140–190	160	5	98–135	115

Fig 2, compiled from the data of Wilson and Byrom, shows the comparatively frequent occurrence of residual hypertension in the presence of a second kidney   In Table II the pooled post-operative pressure readings from all the 34 rats of the present series have been compared with the pooled readings obtained after excising one kidney, but before constricting the
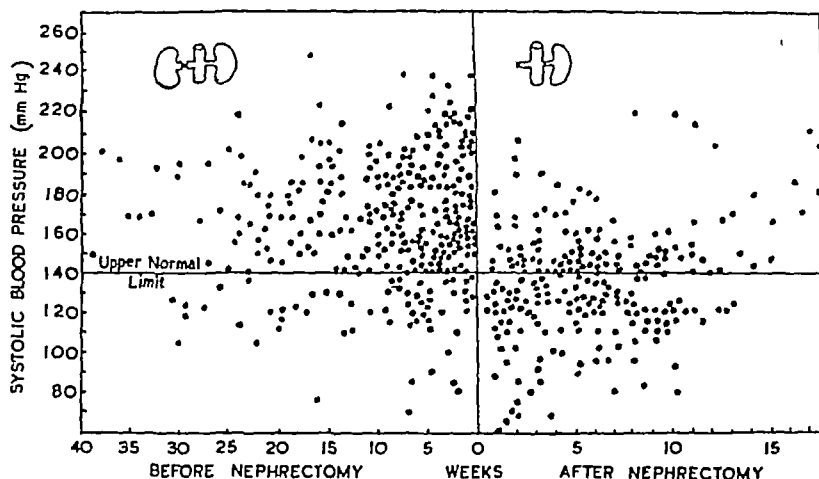
Fig 2   Systolic blood pressure readings from 29 rats with chronic hypertension produced by constricting the artery to one of two kidneys   Showing the effect of excising the ischæmic kidney (or, in 4 instances, releasing the constriction)   For comparison with Fig 1 (from the data of Wilson and Byrom, 1941)

artery to the remaining kidney   The mean difference of 3 mm Hg is negligible   Fig 1, however, reveals several readings which are well above the upper normal limit   These readings were isolated values, obtained on different rats, which could not be confirmed on the same or following days   They were probably due to light anæsthesia, or to over-warming of the rat

TABLE II

Systolic blood pressure readings (ı) after excising one kidney, but before constricting the artery to the remaining kidney in the rat, and, (ıı) after releasing the constriction

Systolic blood pressure	(ı) Before applying clip	(ıı) After removing clip
Number of readings	80	358
Number of rats	80	34
Range (mm Hg)	95–135	70–175
Mean (mm Hg)	114 8	117 8
Standard deviation	10 71	10 14

Closer examination of Table I also shows several instances in which the post-operative readings were consistently higher by from 10 to 20 mm Hg than the normal mean value   We suspect that minor degrees of injury to the renal artery were responsible for these small rises   If they are ascribed, alternatively, to an extra-renal agent, it is obvious that this agent is too feeble and too inconstant to be of either physiological or pathological moment

Symptoms   Parallel with the fall of blood pressure there occurred a dramatic relief of symptoms in those rats from which the clip was removed during a vascular crisis   As a rule the rat had recovered completely by the following morning and this seems significant, because the repair of arterial necrosis is a laborious process measured, not in hours, but in days or weeks (Byrom and Dodson, in press)   It is evident that the cerebral and cardiac symptoms in experimental and, by inference, in human hypertension are due, not directly to arterial necrosis, but to some more readily reversible mechanism

Residual symptoms were observed in three rats   One displayed a permanent hemiplegia and a second violent but brief epileptic convulsions brought on by excitement   In both rats large healed hæmorrhages were found in the brain after death   The third rat had a persistent regular tachycardia, possibly related to scarred cardiac infarcts which were found *post mortem*

Post-mortem findings   The post-mortem findings were in keeping with the ante-mortem observations   No evidence of progressive vascular necrosis was found, but healed or healing lesions were found in the heart and/or the pancreas in 24 of the 34 rats   The kidney appeared normal to the naked eye, apart from inconstant operative adhesions   No microscopic lesions were found in 13 rats and in the remaining 21 the only abnormalities observed were focal changes similar to those described in rats dying before removal of the clip   Finally the heart weight, expressed as a percentage of the body weight was measured and the results were compared with data obtained from rats dying before removal of the clip and from normal young adult rats The data are collected in Fig 3   Considerable cardiac hypertrophy evidently occurred in the hypertensive phase and receded after removal of the clip A few of the post-operative figures are, however, distinctly above the normal range   Possibly the few weeks interval which usually elapsed after the second operation was too short to permit complete involution of the hypertrophied cardiac muscle in these animals   The only exception to the above findings was rat No 19 BC (Fig 1, Table I) in which removal of the clip failed to relieve the hypertension   At post-mortem examination the kidney seemed rather small and on microscopical examination was seen to be enveloped in a layer of hyaline fibrous tissue containing vegetable fibres and foreign-body giant cells   In operating on the rat's kidney, it is our practice to wrap the kidney in a flat layer of cotton wool soaked in warm saline

In this experiment some cotton wool was evidently left adherent to the kidney where it set up a low-grade perinephritis   Artificially induced perinephritis is well known to cause experimental hypertension, and this would obviously not be relieved by removing the arterial clip   This probably accounts for the residual hypertension in an isolated instance
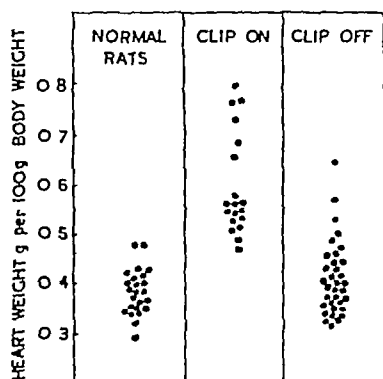
Fig 3   Showing the effect on the cardiac hypertrophy of removing the clamp from the artery to a solitary kidney in the rat with chronic hypertension

DISCUSSION

The above observations show clearly that within the time limits of the experiment, chronic renal hypertension in the rat with a solitary kidney, and the symptoms and lesions attributable to such hypertension, are directly dependent on and co-terminous with the renal ischæmia   This result contrasts sharply with the earlier observation that in rats with a second kidney excision of the ischæmic kidney often fails to abolish the hypertension, or to arrest the progress of arterial disease   The contrast cannot be related to the different means used to remove the primary cause of the hypertension in the two series, namely nephrectomy in the first series and removal of the clip in the present experiments   For in four animals in the former series, Wilson and Byrom removed the clip, leaving the ischæmic kidney in situ, and in all four animals residual hypertension occurred and was, incidentally, followed by the appearance in this kidney of acute hypertensive vascular lesions

Since the present results show that no extra-renal mechanism intrudes in chronic renal hypertension in the rat, it follows that the residual hypertension observed by Wilson and Byrom must have derived from the damaged remaining kidney   Since, as we have shown elsewhere (3), the evidence strongly indicates that hypertension is responsible for this damage, the conception of the vicious circle acquires further experimental support It has long been recognised that widespread renal damage of almost any kind can cause secondary hypertension   Goldblatt's experiments provided a common mechanism for this secondary hypertension , the concept of the

vicious circle in hypertensive renal disease is a logical corollary of the subsequent production of renal damage by experimentally induced hypertension

Clinical implications

Ellis (5) and Wilson and Byrom (16), have fully considered the bearing of the experimental findings on the natural history and classification of hypertensive disease and nephritis   In this discussion we shall restrict our remarks to the question of prognosis   Pending discovery of the cause of essential hypertension, or of empirical means of correcting this cause, the most important practical problem is to define the circumstances in which hypertension becomes irreversible and therefore incurable   From a considerable body of evidence, both clinical and experimental it is now possible to extract a tentative generalisation, namely that *hypertension is an irreversible condition only in so far as it may derive from, or has itself caused extensive irreparable damage involving both kidneys*

Adopting this criterion the common hypertensive diseases of man may be divided for consideration into four groups

1   *Renal hypertension* arising from *bilateral primary disease of the kidney* must be regarded as incurable, but the course may be very chronic and may be favourably influenced by treatment of urinary obstruction or infection

2   In hypertension caused by *unilateral renal disease* removal of the affected kidney may be expected to cure the hypertension provided that significant secondary hypertensive damage has not ensued in the opposite kidney   In the rat, the vicious circle, although common, is by no means an invariable sequel of chronic unilateral renal hypertension   It is, however, important to remember that essential hypertension and unilateral renal disease may arise independently in the same patient   Platt (11) suggests that this may be suspected when there is a family history of hypertension and that in these circumstances nephrectomy may be contra-indicated

3   *Malignant essential hypertension* is such a serious disease that the prospect of cure would seem hopeless but for two facts   Firstly, renal damage is inconspicuous in the early stages of the disease and, secondly, in the experimental animal, we have seen that in the absence of renal damage the removal of the source of the raised pressure is followed by dramatic disappearance of the symptoms, signs and vascular lesions   There are good grounds, therefore, for expecting that early correction of the as yet unknown primary abnormality would be curative

4   *Benign essential hypertension*   In the great majority of these cases the vascular and parenchymatous changes in the kidney are quite different from those of malignant hypertension and suggest a very gradual degenerative process quite distinct from the succession of minute vascular

" explosions " of the latter disease   In the hypertensive rat with one ischæmic and one intact kidney, the disease often runs a benign course, which can be arrested after many months by excising the ischæmic kidney , but in such animals the intact kidney does not present the picture of benign hypertension   It is possible that the lesions of this disease develop too slowly to appear in the short life span of the rat   For this reason no direct light can be thrown on the vital question of whether the damaged kidney of chronic benign hypertension is able to perpetuate the raised pressure   The slow tempo of the process, the rarity of a late " malignant " transformation, and the fact that the renal changes found in chronic benign hypertension are commonly found, unaccompanied by high blood pressure, in extreme old age suggest that the term " benign " is well chosen

SUMMARY

1   An explanation has been sought for the fact that in the rat, chronic hypertension caused by constriction of one renal artery, the opposite kidney being left intact, often persists indefinitely after removal of the ischæmic kidney or release of the arterial constriction (16)

2   It is shown that in the rat with a solitary kidney, the chronic hypertension induced by constricting the renal artery is regularly abolished by releasing the constriction

3   The failure to find evidence of an independent extra-renal pressor mechanism is interpreted as supporting the conclusion that the residual hypertension observed in the rat with a second kidney is maintained by damage inflicted on that kidney by the original hypertension

4   It is suggested that human hypertensive disease should be considered potentially reversible except where it derives from, or has itself caused extensive bilateral disorganisation of the kidneys

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THE CIRCULATORY EFFECTS OF MERCURIAL DIURETICS IN CONGESTIVE HEART FAILURE

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THE value of organic mercurial preparations in the treatment of congestive heart failure is well established, clinical improvement being accompanied by diuresis, loss of body weight, and reduction of œdema   The diuresis is ascribed to the direct action of the drugs on the kidney, whereby re-absorption of water and NaCl in the distal convoluted tubules is diminished (9) Whereas the diuretic effect of these compounds seems to be satisfactorily explained, their action on the cardiovascular system in congestive heart failure has not yet been fully studied   Crawford (1) even suggests that there is no circulatory effect   The observations reported in this paper were made in order to follow hourly the effect of the mercurial compounds on right auricular pressure (R A P), cardiac output (C O), heart rate (H R), blood pressure (B P), and urine output

Clinical Material and Methods

Satisfactory studies were carried out on eleven patients suffering from ischæmic, hypertensive, or rheumatic heart disease   The cases were observed over a period of 5–22 hours   Nine cases were in a severe state of failure with orthopnœa, gross œdema, and grossly raised venous pressure Cases 11 and 13 were in a less severe state of congestion , they were œdematous and, although the venous pressure was not raised on ordinary clinical inspection of the neck veins, cardiac catheterisation revealed that the right auricular pressure was several cm saline above the expected value in such patients   Emphysema, which was present in both, and the propped up position make the expected normal value of the R A P at least 10 cm. below the sternal angle   The cardiac reactions observed were similar to those in the other patients with more obvious congestive failure

Right auricular pressure and cardiac output were measured by cardiac catheterisation (8)   Right auricular blood samples were analysed by the Haldane (3) method   Oxygen consumption was measured by spirometry, and in a number of cases serial records of oxygen consumption were obtained during the experimental period   Right auricular pressure, cardiac output, heart rate, blood pressure and urine volume were measured hourly, except in the first hour when C O and R A P were measured at 10 and 20 min after injection of the mercurial compound

TABLE I

Effects of mercurial diuretics

Case No	Age and sex	Diagnosis	Time	Right auricular pressure cm saline above sternal angle	Cardiac output L per min	% change in cardiac output	Heart rate	Blood pressure mm Hg	Urine output ml per hour	Time from injection to lowest rt auricular pressure Hours	Total urine output up to time of lowest rt auricular pressure Litres	Notes
1	F 77	Ischaemic heart disease A F No response to digitalis	Before During After	+9 +5 +5	2.5 2.3 2.2	— −8 −7	135 144 144	125/? 130/? 130/?	12 540 75	2.0	1.2	Mersalyl 3 c c Severe failure No response to digitalis
2	F 72	Hypertension	Before During After	+11 −2 +1	3.5 4.7 3.3	+25 +4	86 82 80	230/120 185/110 170/90	30 320 25	7.0	3.2	Mersalyl 3 c c
3	F 64	Hypertension, mitral stenosis, aortic regurg, Aur Fib	Before During After	+10 +3 +3	3.5 3.5 2.0	0 −20	80 100 100	200/110 200/110 200/110	50 350 20	5.0	2.4	Mersalyl 3 c c
4	M 61	Hypertension	Before During After	+5 −4 −3	4.5 5.3 4.8	+15 0	90 75 75	200/120 180/110 170/100	75 350 50	6.0	3.0	Mersalyl 3 c c
5	M 60	Hypertension	Before During After	+6 −6 −1	4.5 5.1 4.7	+12 +4	78 84 78	210/125 200/110 175/110	30 300 80	7.0	1.4	Sodium mersalylate 0.2 g

6	M 56	Ischaemic heart disease, complete heart block.	Before	+11	2 5		44	160/?	50	7 0	2 0	Sodium mersalylate 0 2 g
			During	+8	3 9	+36	44	170/?	125			
			After	+10	3 5	+28	44	180/?	50			
8	M 58	Hypertension	Before	+10	2 6		90	210/120	25	5 0	2 0	Sodium mersalylate 0 2 g
			During	+2	3 1	+16	84	200/110	175			
			After	+6	2 8	+7	80	200/110	0			
9	M 60	Ischaemic heart disease Aortic stenosis	Before	+16	3 3		84	120/70	25	7 0	2 4	Sodium mersalylate 0 2 g
			During	+4	3 8	+40	80	130/80	300			
			After	+4	3 1	+20	90	130/80	50			
11	M 75	Hypertension, emphysema Aortic incomp	Before	−3	3 4		84	210/120	—	3 0	0 6	Theophylline free mercuramide 2 c c Observed 6 hrs only
			During	−7	5 0	+32	92	100/100	400			
			After	−2	3 2	−6	02	100/100	180			
12	M 72	Hypertension Ischaemic heart disease	Before	+1	4 0		00	180/?	00	5 0	0 5	Theophylline free mercuramide 2 c c Observed 5 hrs only Pulled out cardiac catheter
			During	+2	5 0	+20	80	180/?	180			
			After	—	—	—	—	180/?	—			
13	M 64	Ischaemic heart disease Emphysema	Before	−5	4 8		80	140/70	—	3 0	1 4	Theophylline free mercuramide 2 c c Restless
			During	−7	5 8	+17	82	130/75	600			
			After	+4	3 3	−43	—	130/75	250			

Before = before injection of mercurial compound

During = during diuresis, at stage when rt auricular pressure was lowest   Cardiac output reached its highest level at this stage

After = at the end of the period of observation, when diuresis had subsided

Cases 7, 10, and 14 were discarded, *see* text

As far as possible the observations were made with the patient in a constant position   In only 3 instances did the patients become unduly restless and unable to maintain a satisfactory position   When this happened the observations were terminated and the data discarded   During the 5 to 22 hr period of observation following the injection of the mercurial compound the patients showed no desire for food and took but little fluid (0 to 2 cups of tea)   Twenty-four hour urinary output was measured for 3 days beforehand   From this the control hourly output was calculated

Measurement of blood volume by the dye method proved unsatisfactory for the purpose of following hourly changes in the blood volume   Trends in blood volume were followed in four cases by hourly estimation of the hæmoglobin levels, using King's cyanhæmatin method (6)
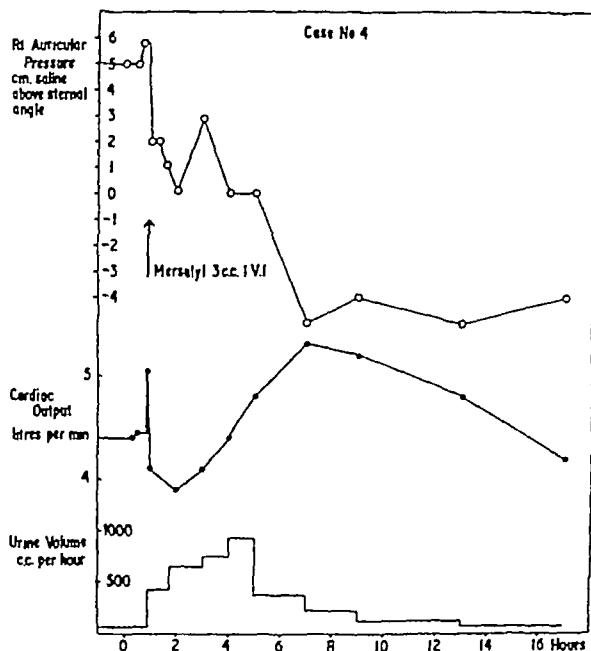
Fig 1   Effect of injectio mersalyh, which contains 5% theophylline on cardiac output (C O), right auricular pressure (R A P) and hourly urine volume (U V), in a case of congestive cardiac failure with œdema   Note initial transient rise in C O and fall in R A.P due to theophylline , slow rise in C O and fall in R A P accompanying diuresis   After 17 hours, diuresis had passed off, C O had returned to initial level, R A P remained down

Mercurial compounds —The first 4 cases were given the British Pharmacopœial mercurial preparation, injectio mersalyh   The dose was 3 c c intravenously   The injectio mersalyh is a 10% aqueous solution of sodium mersalylate with the addition of 5% theophylline   Theophylline stabilises the sodium mersalylate which decomposes in aqueous solution forming toxic substances   Theophylline is stated also to increase the diuretic effect of mercurial compounds in congestive heart failure (1, 2)   It

has, in addition, a well marked though transient cardiovascular action (4)
In the remaining 7 cases, mercurial compounds without the addition of
theophylline were used Since no theophylline-free preparations are
available in this country, a solution of sodium mersalylate was made up by
titration of mersalylic acid with NaOH, pH being adjusted to 7 0 – 7 6 A
freshly prepared solution was used in three cases A smaller dose equivalent
to 2 c c of the B P preparation was employed in order to avoid the risk of
toxic effects In 4 cases, a theophylline-free preparation of a related
compound, *mercuramide, was employed The dose was 2 c c containing
0 18 gms of mercuramide

Results

The results are summarised in Table I, and examples of typical
observations are shown in Figs 1 and 2 These two figures illustrate the
difference observed between the effect of theophylline-containing preparations
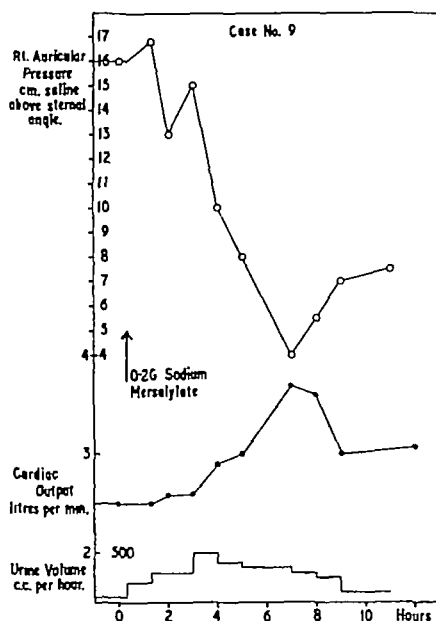
Fig 2 Effect of mercurial diuretic without theophylline Note absence of the initial rise in
C O and fall in R.A.P induced by theophylline containing preparations (compare Fig 1)
slow rise in C O and fall in R.A P accompanying diuresis After 11 hours diuresis had passed
off C O still above initial level, R A P rising but still much below initial level

and mercurial compounds without theophylline With theophylline there
was a rapid fall in R A P and a rise in C O coming on in 10 – 20 minutes,
and passing off within an hour , without theophylline this effect was not
observed This transient lowering of the R A P and rise in C O is comparable
with that described by Howarth, McMichael, and Sharpey-Schafer (4)

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* Prepared specially by Messrs May and Baker

Apart from the theophylline effect, all cases showed a gradual fall in R A P , and, all but two, a slow rise in C O   This began approximately two hours after the injection of the mercurial preparation, and usually reached a maximum in 5 – 7 hours   The highest level of C O coincided in time with the lowest level of R A P and was observed during, or somewhat after the period of maximum diuresis   Towards the end of the period of observation, as the diuresis subsided, R A P showed a tendency to rise, but was still considerably below the initial level when the observations were concluded   C O on the other hand, tended to return to the initial level as the diuresis passed off (Table II)   In only two cases was the increased C O maintained to the end of the experiment   These were cases with very low initial C O   Two cases showed a final level of C O significantly lower than the initial level   In the rest of the cases the final values were comparable with the initial values

TABLE II

Effect of mercurial diuretics on cardiac output

Initial values of cardiac output and percentage deviation from initial values (i) at lowest right auricular pressure and (ii) at end of experimental period

Case No	Initial C O L per min	Percentage change in C O	
		(i)	(ii)
1	2 5	— 8	— 7
2	3 5	+25	— 4
3	3 5	0	—20
4	4 5	+15	+ 6
5	4 5	+12	+ 4
6	2 5	+36	+28
8	2 6	+16	+ 7
9	2 3	+40	+26
11	3 4	+32	— 6
12	4 0	+20	
13	4 8	+17	—43
Mean	3 9	+19	— 1

On the whole there was no significant change in pulse rate or blood pressure, though several hypertensive cases showed some fall in systolic and diastolic pressure at the end of the period of observation

Output of urine   During the first few hours after injection of the mercurial compound, a steady increase in urine output was observed , this was followed by a slow decline to the control level   The average time of

maximum diuresis was 4½ hours after the injection (range 2¼ to 8 hr) In 7 cases followed to the end of the period of diuresis, the control level was reached approximately 10 hr after maximum diuresis   During maximum diuresis the average output of urine was 550 c c per hr (range 300 – 930) The total volume passed from the time of the injection up to the time of maximum effect on R A P and C O averaged 2 0 litres (range 0 5 – 3 2 L)

Discussion

These observations demonstrate the improvement in C O and the relief of venous congestion that accompanies and follows the diuresis induced by mercurial diuretics   By contrast with the compounds containing theophylline, an immediate effect on R A P and C O was not observed following the injection of the mercurial alone

It is of interest to compare the effects of mercurial diuretics and the effects of venesection   The relationship between the changes in R A P and C O from the time of the mercurial injection to the period of maximal circulatory effect is shown in Fig 3 , and in Fig 4 is reproduced, for comparison, a similar diagram from Howarth, McMichael, and Sharpey-Schafer's paper on venesection (5)   The general slope of the lines relating change in R A P to change in C O bears a close resemblance in the two figures   It is evident, therefore, that the circulatory effects of mercurial diuretics in congestive heart failure are comparable with those induced when R A P is lowered by mechanical means, i e , by venesection or the application of venous constricting cuffs to the thighs   The difference lies in the time interval elapsing between the procedure and its effect—in the former case, a matter of hours, in the latter, of minutes

It is thus probable that the rise in C O is accounted for by reduction in R A P   Reduction in R A P of the order observed in our cases, was induced by Howarth, McMichael, and Schafer (5), by withdrawal of 450 – 1,000 c c of blood   Such a reduction in circulating blood volume may occur in the course of the diuresis induced by mercurials   We were unfortunately unable to obtain satisfactory confirmation of this   Significant hæmoconcentration was observed in 2 of 4 cases in whom hæmoglobin levels were followed, but three of these four were cases in which the observations were cut short owing to the restlessness of the patient, and no valid comparison between the R A P and hæmoconcentration was possible   In normal subjects, Lyons and others (7) using the dye method, claim reductions in plasma volume of up to 15%, 9 to 12 hours after mercurial injections Further work is clearly necessary before this matter can be settled   Warren and Stead (10) have suggested that the maintenance of high venous pressure in congestive failure may be partly accounted for by raised tissue tension due to œdema   Reduction in œdema cannot be held to account for
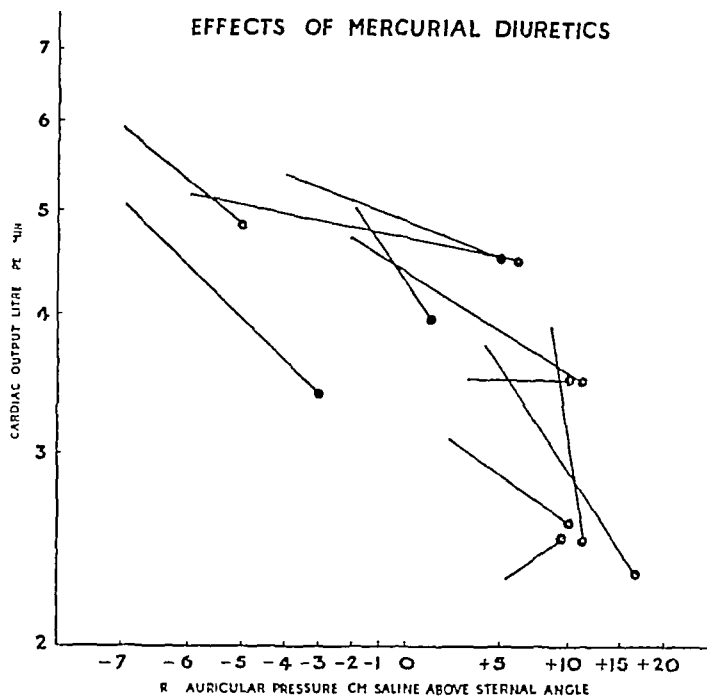
Fig 3   Effect of mercurial diuretics on right auricular pressure and cardiac output, plotted on logarithmic scales   The logarithmic scale for right auricular pressure is arbitrary in Figs 3 and 4, and zero represents the sternal angle   Black dots show initial data and lines are drawn to the maximal circulatory responses
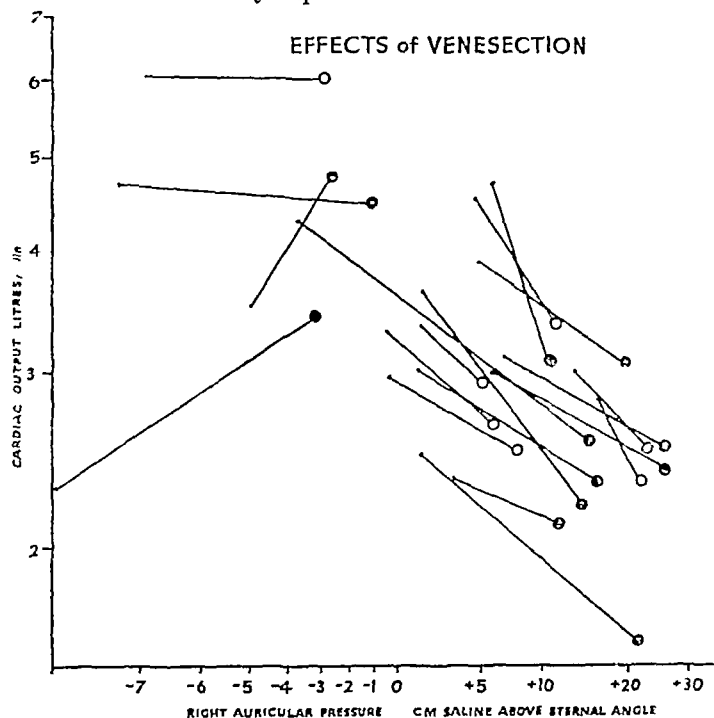
Fig 4   (After Howarth, McMichael and Sharpey Schafer, Clinical Science, 1946 47, 6, 41) Effect of venesection (black circles) and cuffs (white circles) plotted on the same scale as Fig 3   The response to mercurials and venesection is similar

reduction in R A P in the early stage of the experiment, but may play a part in keeping down the R A P towards the end of the period of observation, when reduction of body fluid is maximal

Finally the possibility should be held in mind that organic mercurial compounds, which after all are powerful drugs capable in rare cases of causing sudden death, may have some direct action on the cardiovascular system, possibly lowering venous pressure, in a manner analogous with digoxin

Summary

1   The circulatory effects of I V injections of mercurial diuretics have been studied in 11 cases of congestive heart failure

2   A rise in cardiac output and a fall in right auricular pressure was observed   These effects began about 2 hours after injection of the mercurial preparation, reached a peak in 5 to 7 hours, and tended to pass off with the return of urine output to normal

3   Mercurial preparations containing theophylline such as mersalyl caused, in addition, an initial but transient rise in cardiac output and fall in right auricular pressure which was not observed after preparations not containing theophylline

4   The significance of these circulatory changes is discussed

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A NEW METHOD OF CLINICAL SPIROMETRY

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THE ordinary spirometer from which oxygen or an oxygen rich mixture is rebreathed is well suited for the determination of oxygen uptake but is limited in its application to more general respiratory problems   Not only is it impossible to study the response to CO_2, low oxygen tensions and other mixtures of gases, but also the inspired air, with its high and changing oxygen content, is abnormal and may alter the respiratory pattern by relieving anoxia   Some of these drawbacks can be overcome by running oxygen into the air filled spirometer at the rate of uptake (2), but this method is limited in its use and requires the subject to be in a constant and relatively basal condition   At present, if continuous respiratory tracings on air or any mixtures of gases is required, only two methods are available   The plethysmographic method in man is cumbersome and technically difficult, while the recording of pressure changes in tubes fastened round the chest is neither sensitive nor volumetric   The method described in this paper allows an accurate volumetric tracing to be obtained while breathing air or any mixture of gases, using, as far as the patient is concerned, only a mouthpiece and nose-clip as in standard spirometry   The whole apparatus is incorporated in a trolley and can be used at the bedside at short notice

Method

The method has been developed by the authors from a simpler plethysmographic technique used previously by Christie (1)   If a subject breathes from a rigid air-tight box to which a spirometer is connected, it is obvious that the spirometer will rise and fall with inspiration and expiration If a bag (Fig 1, B) is introduced into the box (X) with an inspiratory tube leading from bag to mouthpiece (M) and an expiratory tube connecting mouthpiece to spirometer, which in turn is connected with the box, then the gas to be inspired, and that expired are segregated within a rigid system which is attached to the spirometer   Gas is withdrawn from the bag (B) on inspiration and expired air is returned to the box, outside the bag through the spirometer, which will record faithfully the respiratory excursions of the subject   By the introduction of many-way taps a number of these boxes can be employed using the same spirometer and the subject can be switched from one gas to another, a continuous respiratory tracing being obtained

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The boxes are of light metal with reinforcing struts incorporated in the wall   Their capacity is 100 litres   The bags are made of light rubberised gaberdine and will hold 80 litres   All connections are of wide bore rubber tubing and non-return rubber spear valves are inserted on each side of the mouthpiece (V)   If desired, an external carbon dioxide absorbent canister
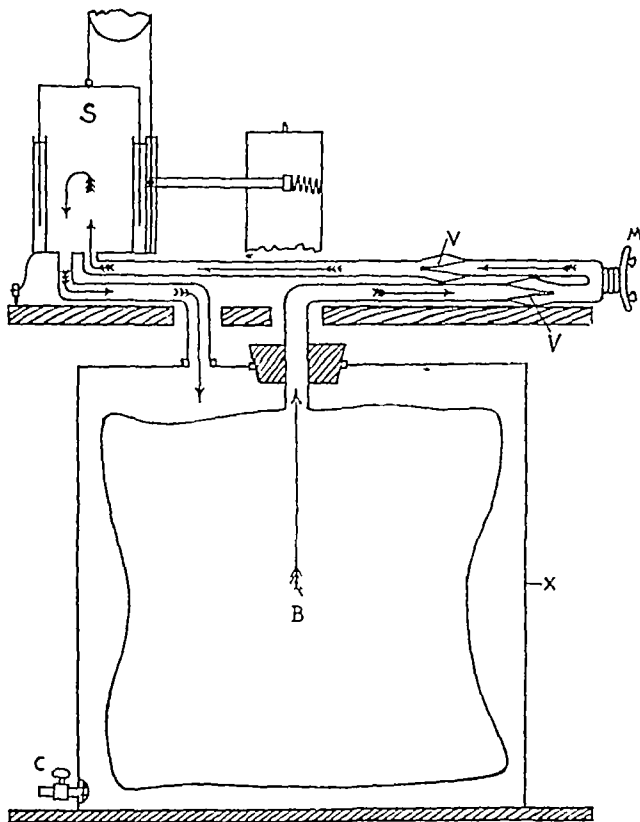
Fig 1   Diagram of circuit employed   S —Recording spirometer   M —Mouthpiece   V — Non return rubber spear valves   B —Bag of 80 litre capacity and box of 100 litre capacity   C —Stopcock   Direction of air flow shown by arrows

can be placed between the mouthpiece and spirometer   The total cost of one box and bag is about a quarter that of a standard spirometer   A six or eight litre Benedict spirometer is used, and this with two boxes incorporated in a trolley is shown in Fig 2   With suitably placed taps, the circuit can be changed so that the patient breathes from either of the bags or from the spirometer alone, and the change can be executed so quickly and quietly that the patient is unaware of it and the respiratory tracing is uninterrupted

The circuit is larger than that in a standard spirometer and the resistance to inspiration is slightly greater as gas is moved through practically the whole circuit   This causes the inspiratory resistance to be about 0 4

cm water (Minute volume 5-10 litres) more than that of the spirometer and
non-return valves employed   Since the exhaled gas is totally accommodated
by the spirometer the expiratory resistance is not increased   Many
extremely debilitated and dyspnœic patients have breathed from the
apparatus without discomfort and have at no time complained of any
resistance   The spear valves, despite their handiness and reliability are
to be substituted by lighter non-return valves as they are responsible for
most of the resistance of the apparatus

The bag can be filled with any mixture of gases   It is first completely
emptied by increasing the pressure within the box by blowing air through
a small side tap (C, Fig 1)   This tap is then connected to a flowmeter
and gases are run from cylinders into the bag   The volume of any gases
introduced into the bag is thus measured by air displacement and CO_2 or
any gas mixture can be measured accurately (to 0 1%) using one standard
air meter   This displacement technique makes what can be a very difficult
procedure into a simple and rapid process   The space between bag and box
must be washed out with air before mixing, as the presence of any soluble
gas will falsify the flowmeter reading   The gases are passed through warm
water on the way to the bag , this causes near-saturation with water vapour
and subsequent cooling to room temperature and condensation in the bag,
leaves the gas saturated   Dry gases cause the subject discomfort after a
few minutes, and the constant addition of water vapour by the patient's
lungs to the circulating gas causes a small but definite increase of volume of
the expired air   Where standard mixtures of gas are employed the bags
are usually filled from the prepared cylinders, but it is of advantage to be
able to make any mixture that may be required   After filling the bag,
the apparatus is left for 15 minutes so that its contents attain room
temperature   The apparatus should be used within two hours of filling
otherwise slight changes of concentration will take place owing to diffusion

As equilibrium between cooling and heating which occurs in the Benedict
spirometer might not be expected in so large a circuit, errors from this source
were anticipated   However, careful testing showed that, owing to the length
of tubing, the size of the box and the passage of the expired air over the water
seal of the spirometer, there was no significant rise of temperature in the
box which remained at room temperature

The inertia of the spirometer is the only factor which leads to a
measurable error in this method   The spirometer will not start to move
until sufficient negative pressure is developed to overcome the resistance of
the circuit, and it will therefore only record the tidal air minus the volume
of air whose removal from the box will cause this negative pressure   This
will lead to a slight " damping " of the excursion on the respiratory tracing,
but the base line will remain volumetrically accurate (other things being
equal) and will give an accurate indication of the gases absorbed, retained,
or metabolised   This " damping " remains constant and does not vary

with the tidal air nor with the speed of respiration The volume of this "lag" can be determined by using another carefully calibrated spirometer as an artificial lung In the 100 litre capacity box, this error is about 10 c c per excursion, and, in most experiments, this can be ignored If a larger box is used this factor becomes more significant and for this reason when a tracing over a long period is desired, a series of 100 litre boxes are used

If volumetric estimations such as oxygen consumption or gas absorption are being carried out, any change of atmospheric temperature or pressure would, in itself, cause a volume change This factor has been carefully checked and found to be of no significance providing the precaution is taken to avoid using the apparatus soon after transference from one room to another of considerably different temperature

Leaks can be tested for in the usual manner by weighting the spirometer and connecting it, in turn, with all parts of the circuit

Experimental

If there is no CO_2 absorbent canister in the circuit, the expired gas will be returned to the spirometer and box unchanged, and the tracing will slowly rise (the spirometer bell falling), as slightly less carbon dioxide is produced than oxygen used If the respiratory quotient is exactly one the tracing will be level, and if above one the slope of the tracing will fall Thus changes in the respiratory quotient can be observed from the tracing without gas analysis

If changes in ventilation with different gases are being investigated it is important that the resting minute volume while breathing air should first be determined Even unapprehensive patients may respond to any unusual "respiratory environment" by an increase in ventilation although their respirations remain serene and regular Du Bois (3) has emphasised that this may invalidate many experiments and that slight over-ventilation is impossible to detect by present methods for this reason the precaution has been usually taken of training subjects in the experimental procedure, repeating the experiment a number of times, and only accepting the minute volume on air when it has been constant over a number of minutes With the present method, however, any hyperventilation will declare itself by a rise in the respiratory quotient, and the resultant sloping down of the tracing The minute volume often takes several minutes to settle to a steady figure and this can be easily assessed from the respiratory record This technique makes "practice runs" unnecessary with most subjects

Fig 3 shows a typical chart of a normal subject breathing first air and then 4% CO_2 It will be noted that when 4% CO_2 is breathed the level of the tracing rises owing to the retention of carbon dioxide in the body

When an equilibrium between the tissues and the inspired CO_2 is established the level of the tracing again becomes stabilised, so that the amount of CO_2 retained can be measured
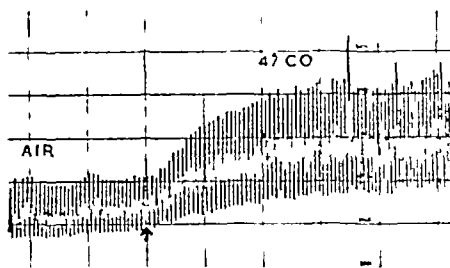
Fig 3   Reaction of normal subject to 4% CO   Subject breathing air until signal 4% CO and air after signal   No CO absorbent in circuit   Tracing reads from left to right, vertical lines mark one minute intervals and space between horizontal lines 500 c c

Fig 4 shows a normal subject breathing air and then 12% oxygen in nitrogen , the increased ventilation caused by anoxia washes out carbon dioxide with a resultant rise of the respiratory quotient   The demonstration of this phenomenon confirms the sensitivity of the apparatus to even slight degrees of over-ventilation
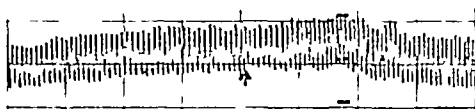
Fig 4   Reaction of normal subject to 12% oxygen in nitrogen   No CO absorbent in circuit (otherwise legend as in Fig 3)

The circuit without the canister can be used for many other purposes Tracings of patients with abnormal respiratory behaviour can be recorded while breathing air and the effect of carbon dioxide, oxygen and anoxia ascertained
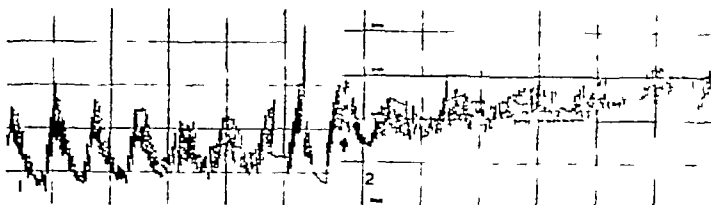
Fig 5   Cheyne-Stokes breathing in a case of malignant hypertension
    1   Breathing air   No canister   2   Breathing oxygen   No canister   (There is an interval of few seconds between 1 and 2)   Signal marks change over from air to oxygen.

Fig 5 shows two such tracings in a case of malignant hypertension with Cheyne Stokes breathing   The marked periodicity while breathing air and a reduction of this periodicity when breathing oxygen is clearly seen   A

repetition of these tracings with an absorbent canister in the circuit showed
that the shift of respiratory level is a true one and not due to changes in the
respiratory quotient
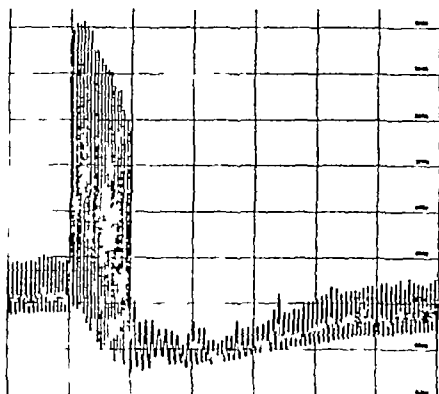
Fig 6   Normal subject breathing air before, during and after hyperventilation   No CO$_2$
absorbent in circuit

Tracings of the vital capacity and of hyperventilation or apnœa and
their after effects, can be obtained while breathing air or any other mixture
of gases   In a normal individual maximal hyperventilation causes consider-
able washing out of carbon dioxide followed by decreased ventilation and,
usually, periodicity   All these phenomena can be seen in Fig 6
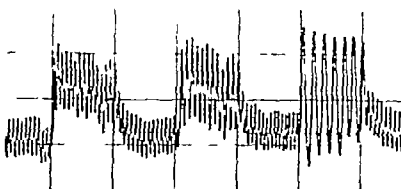
Fig 7   Emphysematous subject making three separate attempts to hyperventilate while
breathing air   No CO$_2$ absorbent in circuit

Emphysematous patients give a very different record , the respiratory level
is raised while attempting to hyperventilate and these patients are usually
incapable of washing out any significant amount of carbon dioxide (Fig 7)
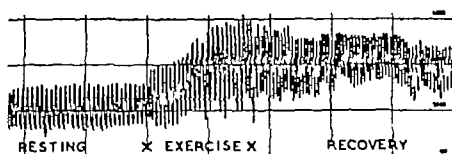
Fig 8   Emphysematous subject breathing air before, during and after mild exercise   No CO$_2$
absorbent in circuit

The effect of exercise on the respiratory quotient can be studied   Fig 8
shows a case of emphysema breathing air, first while at rest, then during
mild exercise, and finally during the recovery period   Carbon dioxide
was still being washed out eight minutes after the period of mild exercise

The apparatus is also useful for volumetric respiratory tracings on air when alveolar samples are being taken   An 'alveolar trap" can be easily incorporated into the circuit   It is then possible to assess from the record the significance of such factors as regularity of breathing, depth of expiration at time of sampling, and prolongation of expiration due to emphysema or spasm
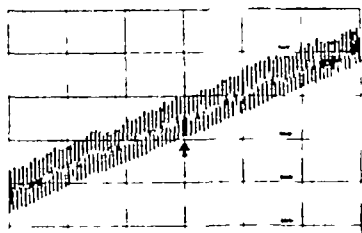
Fig 9   Oxygen uptake of normal subject in resting state as determined by Benedict s spirometer and by box spirometer while breathing air (CO₂ absorbent in circuit)   Signal marks change over from oxygen to air

Oxygen uptake under various conditions

If an absorbent canister is inserted between the mouthpiece and the spirometer the oxygen uptake is accurately recorded provided there is no carbon dioxide or highly soluble gas present in the inspired air   Thus the metabolic rate can be determined while breathing air as easily as when breathing oxygen   Fig 9 shows, first, the rate of oxygen uptake as measured
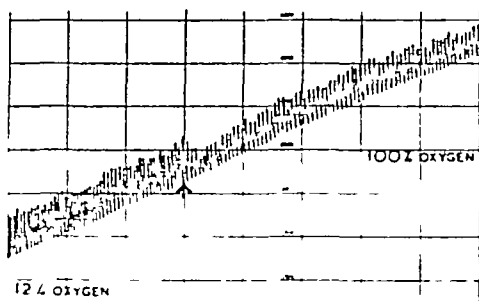
Fig 10   Oxygen uptake and ventilation of normal subject when breathing 12% oxygen and 100% oxygen. CO absorbent in circuit   Signal marks change over from 12% oxygen to 100% oxygen

by an ordinary oxygen filled Benedict spirometer   At the signal the subject started to breathe air from the box circuit and it will be seen that the rate of oxygen uptake remains unaltered   Similarly it can be shown that the oxygen uptake remains unchanged while the subject is breathing 12% oxygen, although there are marked changes in ventilation (Fig 10)   Changes in metabolic rate with exercise can also be measured, while breathing various

tensions of oxygen   Fig 11 shows a subject working steadily at the rate of 300 kilogramme-metres per minute while breathing air, 15% oxygen and 12% oxygen   A change from air in one box to air in another causes no change, a change from air to 15% oxygen causes a slight increase in ventilation only (25 3 to 30 litres p m), but when 12% oxygen is breathed there is a dramatic fall in oxygen uptake and rise in ventilation   In the last experiment the

(a)   (b)   (c)
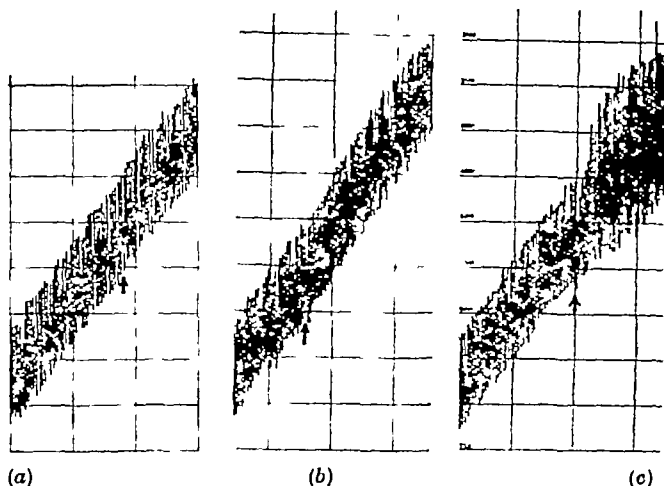
Fig 11   The effect of changes in oxygen tension on the oxygen uptake and ventilation of a normal subject breathing air at constant rate of work (300 kg m per min)   (a) Air → air (b) Air → 15% oxygen   (c) Air → 12% oxygen

subject was cyanosed and anoxic and the experiment had to be discontinued This technique of lowering the oxygen tension during exercise has been used in pathological states as it allows critical changes in ventilation and oxygen uptake to be observed without violent maximal exertion

Absorption of inactive cases

If a subject first breathes air and then a mixture of oxygen and nitrous oxide, the tracing shows the absorption of nitrous oxide volumetrically This can be demonstrated with or without the absorbent canister in the circuit   The former is preferable as changes in level due to variation in the respiratory quotient will not be superimposed   In Figs 12 and 13 the subject first breathed air and then 25% and 50% nitrous oxide with no absorbent canister in the circuit   The amount of nitrous oxide absorbed with each of the concentrations can be seen and in Fig 12 the rate of its elimination while breathing air is shown   Following three minutes exposure to 50% nitrous oxide the subject became completely unaware of his surroundings and held his breath for some time   After this, although the respirations were irregular and the total ventilation reduced the rate of absorption increased   This was probably due to the apnœa and excitement of the first stage of

anæsthesia causing an increase in cardiac output although changes in the respiratory quotient may also be involved 25% nitrous oxide usually causes some dissociation and feelings of unreality, but 15% and 20% do not cause these disturbances When breathing the lower concentrations, total quantities of gas can be absorbed without ill effects that would cause unconsciousness if breathed in higher concentrations
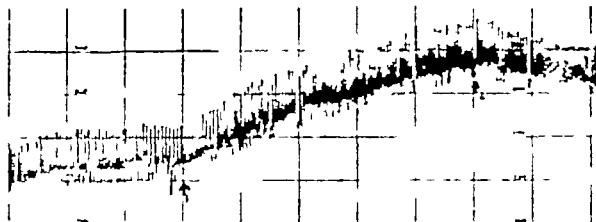
Fig 12 Normal subject breathing air 25% nitrous oxide in oxygen followed by air No CO₂ absorbent in circuit First signal marks change over to nitrous oxide, second signal return to air
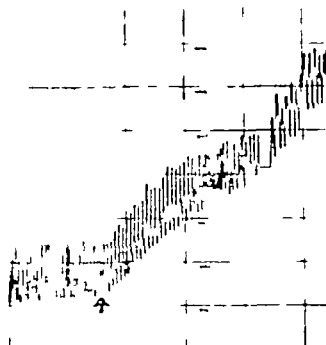
Fig 13 Normal subject breathing air and 50% nitrous oxide in oxygen No CO absorbent in circuit Signal marks change over from air to nitrous oxide

SUMMARY

A new and simple method of spirometry is described which enables volumetric respiratory tracings to be obtained while breathing any mixture of gases

The inertia, resistance and temperature changes in the circuit and technique of filling the spirometer are described

Some uses of the spirometer in clinical investigation are given If no CO_2 absorbent is employed the reaction to CO_2 and anoxia, changes in respiratory quotient the vital capacity and abnormal respiratory behaviour breathing different gases can be studied With an absorbent canister in the circuit, the oxygen uptake and ventilation can be determined while breathing air or any other percentage of oxygen The spirometer also records the absorption of soluble inactive gases

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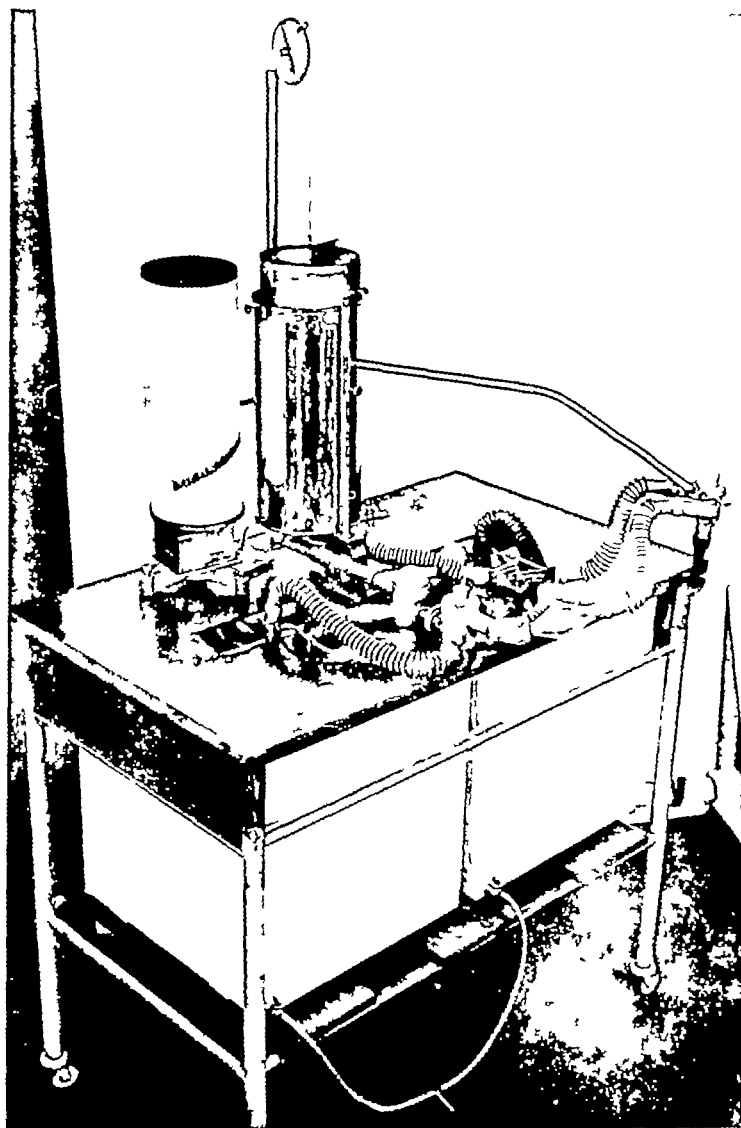
Fig 2 Spirometer with two box bag circuits incorporated into ward trolley Fourway taps are shown which enable subject to breathe from either bag or spirometer alone

THE RESPIRATORY RESPONSE TO CARBON DIOXIDE AND ANOXIA IN EMPHYSEMA

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In 1920 R W Scott demonstrated that two patients with chronic pulmonary emphysema showed little reaction to the inhalation of increased percentages of carbon dioxide (7) He related this to the increased buffering action of the bicarbonates and the inability to increase adequately the tidal air and respiratory rate owing to the faulty mechanics of the chest In this investigation the phenomenon is studied more extensively in a larger group of cases with adequate controls

Method and selection of cases

The patients investigated all complained of shortness of breath and were referred to us as definite or possible cases of emphysema A number of cases were rejected with other diagnoses such as tuberculosis, carcinoma of the bronchus, or proven myocardial disease If a patient had only moderate signs of emphysema but no other cause of the dyspnœa, he was included in the series A few cases of asthma who were complaining of shortness of breath between the attacks were also included In the history particular note was made of cough dyspnœa, asthma and capacity for work, and a full clinical examination was carried out, the respiratory and cardiovascular systems being examined with particular care The degree of bronchospasm was assessed by auscultation at rest, during attempted hyperventilation and after exercise Dyspnœa was also noted after graduated exercise An electrocardiogram and X-rays were obtained, plates being taken at 6 feet in full inspiration and full expiration in the antero-posterior and lateral positions The plasma CO_2 combining power was also determined, venous blood being drawn without stasis and oxalate used in minimal quantities The specimen was centrifuged immediately and the plasma pipetted off at once and equilibrated with CO_2 at 40 mm Hg pressure at room temperature The manometric method of Van Slyke was used The vital capacity was measured with a Benedict's spirometer, the subject being in a semi-recumbent position Four estimations were carried out and the largest volume noted, as by far the most important variant appeared to be the degree of effort and co-operation The vital capacity was converted to a percentage of " normal " using West's formula (10) It is realised

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that this is far from accurate but the important factor of body size is allowed for and therefore it was considered that such a conversion was necessary Alveolar sampling was performed in most cases but it was often impossible to obtain a satisfactory sample and repeatability was very poor   In view of these difficulties these figures are not given

The method already described (4) was used in determining the response to CO_2 The subject lay supine for at least twenty minutes to achieve a steady respiratory state (9) , a tracing while breathing air for about six minutes was then obtained and was followed by a tracing while breathing 4% CO_2 for five minutes (Fig 1)   4% CO_2 was employed for a number of reasons   This concentration causes no disturbance in normal or abnormal individuals (unless acidosis is present) and it allows an exposure of 5 minutes or more using an 80 litre bag   It was considered that the factor of mechanical limitation of ventilation would be reduced to a minimum by using a moderate concentration of CO_2
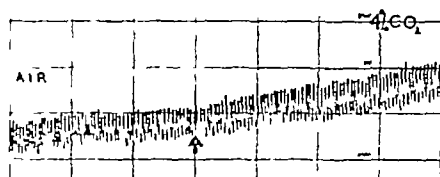
Fig 1   Patient with emphysema breathing air and 4% CO_2 (Signal marks change over —
Tracing shows little change in ventilation and should be compared with tracing from normal
subject shown on page 25 (4))

The reaction of a control group of 28 normal individuals was also determined   They were of both sexes and their ages varied between 18 and 59 years   All were in good health without dyspnœa, and there was no history of lung disease, heart disease, or of nephritis

Results

The reactions of the normal series to the first five minutes of exposure to 4% CO_2 are shown in Fig 2   The maximal increase of minute volume, stated as a percentage of the minute volume while breathing air, was taken as the significant figure (Fig 3)   In the large majority of subjects the ventilation was maximal in the fifth minute, but in a few subjects this occurred in the third or fourth minute of exposure   The mean minute volume of the group (7 05 litres) tallied well with the figures given by Shock and Soley (8, 9)

Of the 30 patients who were investigated, 23 were considered to have mild to severe emphysema and 7 to be asthmatic without definite emphysema The age, minute volume, reaction to CO_2, and plasma CO_2 combining power of these two groups are shown in Table I   The history, degree of dyspnœa, physical signs of emphysema, chest measurements, cardiovascular and X-ray findings are summarised in Table II   Asthma is only noted when the patient had classical attacks, but if bronchospasm was found on examination,

or the history suggested its being an important factor, it is noted under a
separate heading   The degree of dyspnœa was assessed as follows —

Degree 1   Dyspnœa noticed by the patient but confined to severe exertion

Degree 2   Dyspnœa on walking rapidly or climbing flight of stairs

Degree 3   Dyspnœa on walking at moderate speed on the flat

Degree 4   Dyspnœa on the slightest exertion (*e g* undressing)

Degree 5   Dyspnœa at rest
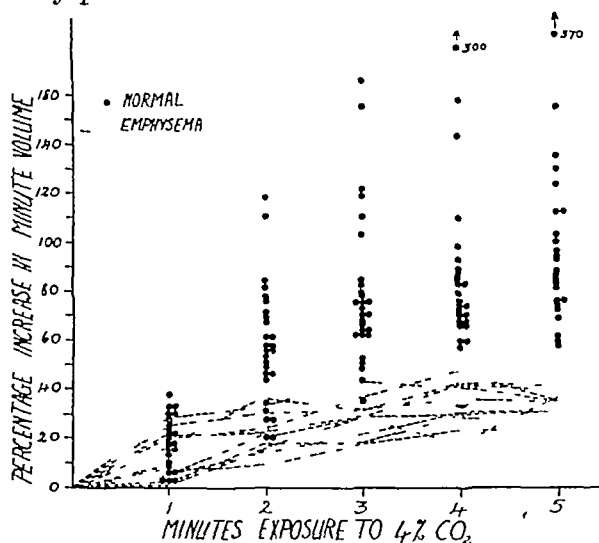
Fig 2   The increase in ventilation of 28 normal subjects during the first five minutes of exposure
to 4% CO   The reaction of the ten most tolerant cases of emphysema is also shown
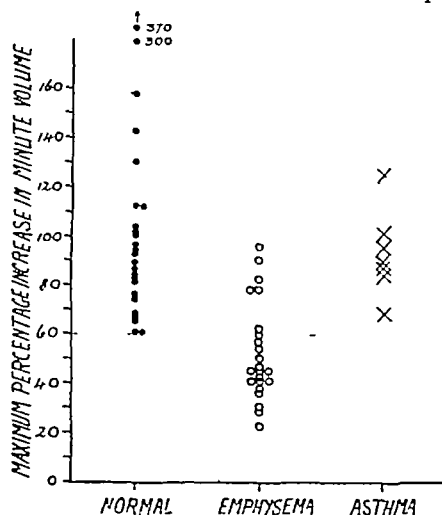
Fig 3   The maximal increase in minute volume in the first five minutes of exposure to 4% CO
in 28 normal, 23 emphysematous and 7 asthmatic subjects

TABLE I

Age, reaction to 4% CO_2 and CO_2 combining power in 23 cases of emphysema (1 23) and 7 cases of asthma (24 30)

Case No	Age Yrs	Min vol air (c c)	Percentage increase minute volume breathing 4% CO_2					CO_2 capacity venous plasma
			1st min	2nd min	3rd min	4th min	5th min	
1	23	5,410	8	9	16	23	23	77 1
2	55	8,150	21	22	29	29	24	60 0
3	49	6,620	4	16	21	29	31	74 2
4	36	7,000	2	15	29	17	37	73 0
5	48	7,667	7	22	33	36	39	70 1
6	42	9,560	16	36	32	42	35	72 2
7	61	7,215	1	18	18	30	42	77 7
8	57	7,180	25	30	32	42	41	69 0
9	60	7,830	28	33	43	41	40	68 5
10	69	10,420	20	24	37	46	35	70 5
11	66	10,030	7	15	40	44	46	71 1
12	52	9,017	11	27	30	46	46	62 4
13	59	7,400	11	30	47	22	34	68 8
14	45	8,716	16	27	19	32	51	66 5
15	54	8,010	15	22	32	55	54	68 0
16	57	7,350	6	34	43	58	52	67 0
17	61	8,000	26	37	42	56	61	69 3
18	53	8,340	26	54	63	59	63	74 8
19	52	8,610	26	63	76	79	78	67 0
20	50	8,680	17	50	44	61	79	72 2
21	48	6,570	30	74	65	62	83	66 0
22	56	7,580	15	43	61	85	91	60 0
23	55	6,630	30	64	84	78	96	68 1
24	55	3,862	26	83	98	102	126	60 6
25	18	7,070	1	49	60	78	102	57 8
26	27	5,830	13	40	60	53	96	56 2
27	30	9,480	12	52	66	90		57 5
28	24	6,073	37	56	62	77	86	59 5
29	30	9,107	27	55	47	62	70	70 2
30	43	10,030	23	63	77	78	89	68 2

It will be noted that 16 out of 23 cases considered to have emphysema gave reactions in the first five minutes of exposure to 4% CO_2 that were clearly below the normal range (Fig 3) The physical signs of emphysema in the 7 patients giving a normal reaction to CO_2 were, on the whole, less than in the others on this group, although two of them had gross signs of the disease The degree of dyspnœa in the seven normal reactors was however significantly less than in the rest of the group (Table II), and three had vital capacities of 70% or more of normal, whereas only one out of the 16 CO_2 tolerant cases reached this figure The alkali reserve of all cases was within the normal range although most CO_2 tolerant cases had high alkali reserves within this range (Table I)
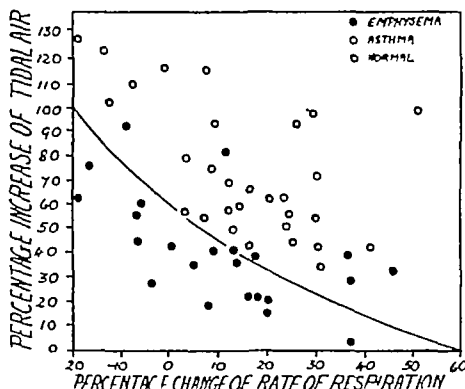
Fig 4 The percentage increase in tidal air plotted against the percentage change in respiratory rate when breathing 4% CO_2 in normal, emphysematous and asthmatic subjects The curve represents a 60% increase of minute volume and therefore separates the normal reactors from those with impaired reactions to CO_2

In Fig 4 the percentage increase in tidal air during the minute of maximum reaction is plotted against the percentage change in respiratory rate It will be seen that most normal subjects increased their tidal air by 40 to 70% and their respiratory rate by 10 to 30% A few subjects with a marked increase in tidal air (over 100%) actually slowed their rate of respiration The emphysematous subjects showed no significant difference in the type of reaction and a number increased their tidal air or respiratory rate normally although the total ventilation was markedly impaired It would appear, therefore, that inability to increase the tidal air or respiratory rate is not a fundamental cause of the impaired reaction to carbon dioxide

The group of asthmatic patients all gave a normal reaction during the first five minutes of exposure to 4% CO_2 (*see* Table II and Fig 3) Cases 24 to 28 were women and did not show any tendency to deepening of the chest , in contrast all three male asthmatics had some deepening of the chest but otherwise no convincing signs of emphysema The vital capacity of the asthmatic group was considerably higher than in those cases classified

TABLE II

Hıstory, degree of dyspnœa, physıcal sıgns of emphysema, chest measurements, cardıovascular and X ray findıngs, and vıtal capacıty ın 23 cases of emphysema and 7 cases of asthma

Emphysema

Case No	Years bronchitis	Years asthma	Degree of dyspnoea	Broncho spasm	Physical signs of emphysema	Ant postr and latl mens (cm)	Blood pressure	E C G	Emphysema X ray	% normal VC
1	20	12	5	Sl	++	23/23	104/78	Class cor pul	++	37
2	20	0	4-5	Cons	++	24/26	128/98	P₂+	+	40
3	20	20	3	Cons	Mod	22/25	150/90	P₂+	Sl	39
4	33	10	4	Cons	++	25/23	130/80	R A D	++	64
5	10	0	3	+	++	28/27	140/90	R A D	++	56
6	20	0	2	+	+	25/27	130/80	P₂+	++	54
7	5	0	4	Sl	+	20/27	110/75	P₂+	+B*	61
8	28	0	5	Sl	+	27/27	130/80	N a d	+	64
9	30	4	5	++	++	26/23	114/72	P₂+R A D	++B	34
10	3	0	3	0	++	27/25	215/100	Flat T₁	Sl	44
11	1	45	4	Sl	++	28/27	120/70	L A D (St)	Sl	54
12	20	1	4	Cons	++	26/23	118/75	P₂+	+	62
13	27	0	3	Sl	++	24/25	200/95	N a d	++	45
14	1	0	2-4	++	Mod	27/28	128/65	N a d	+	45
15	30	0	2-3	Cons	Mod	22/28	135/100	P₂+R A D	Sl	71

16	15	1	3	Cons	++	28/26	154/84	P_2+RAD	++	40
17	7	2	3	++	Mod	23/20	165/95	LAD	S1	48
18	27	7	2-3	++	+	21/28	138/95	LAD	+	58
19	2	2	2	0	S1	29/29	124/96	Flat T_1	0	70
20	2	1	3	++	++	25/25	85/55	P_2+	+	43
21	20	20	1-3	+	++	30/26	120/65	Nad	+	44
22	1	0	1-2	0	S1	21/25	98/70	P_2+	S1 B	72
23	28	0	2	Cons	Mod	23/25	135/90	P-+	Mod	88
Asthmatics										
24	2	28	2		S1	17/22	160/90	LAD	S1	50
25	0	17	1		S1	21/26	125/75	P_2+	0	70
26	0	15	1		Nil	20/25	115/80	Nad	0	88
27	8	17	2		Nil	19/25	95/95	LAD	0	84
28	8	19	1		S1	26/26	100/70	Nad	0	61
29	8	8	2		S1	23/25	135/80	RAD	S1	55
30	5	8	2		S1	26/29	105/85	Nad	S1	79

* Bullae

as emphysema   Two patients showed bronchospasm almost continually between the acute attacks, and four had severe bronchospasm (as judged clinically) when their reaction to CO_2 was determined although this did not impair the capacity to increase their ventilation   Only one asthmatic found the increase in respiratory velocity distressing and the experiment had to be discontinued after the third minute   The changes in respiratory rate and tidal air in this group did not differ in any way from that of the normal subjects

Longer exposures

It was reported by Dripps and Comroe (5) that when 42 normal individuals breathed 7 6% CO_2, only 27 subjects reached a constant minute volume in 2½ to 8½ minutes   For this reason some of the patients showing
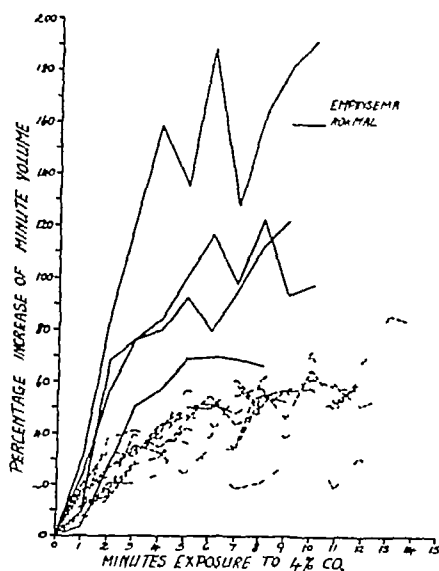
Fig 5   The response of nine patients with severe emphysema to more prolonged exposures to 4% CO_2 The response of four normal subjects is also shown

impairment of ventilation in the first five minutes of breathing 4% CO_2 were submitted to longer exposures   Nine patients with severe emphysema were tested, the length of the exposure being 12 to 14 minutes , four achieved an increase of respiratory minute volume within the normal range of reaction to 4% CO_2 (8) in nine to twelve minutes and four achieved nearly normal percentages (Fig 5)   The remaining subject still showed extreme impairment of ventilation after thirteen minutes exposure (28% increase)

After this experiment each case ventilated on air to his maximum ability   Seven of the nine cases were capable of hyperventilation (70% to 117% increase of minute volume) that was greater than that produced

by 4% CO_2 and well within the normal range of increase   The patient
with a prolonged impaired reaction could only increase his minute volume
by 40%

Repeatability

The response to 4% CO_2 was repeated on a number of cases, mostly
on those who showed impaired reactions in the first five minutes of exposure
The results are shown in Fig 6   In the large majority of instances, there was
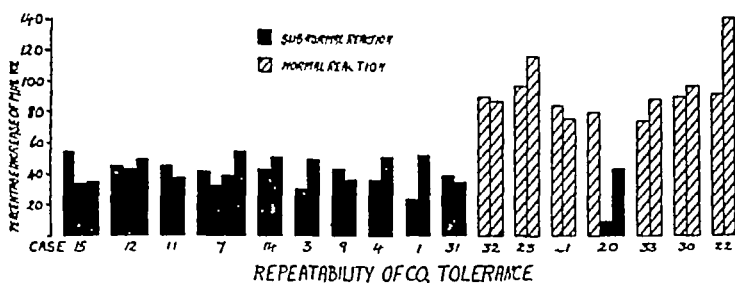
Fig 6   Repeatability   The maximal reaction during five minute exposures to 4% CO_2 in abnor
mal subjects on successive occasions

an interval of several months between the experiments   Repeatability was,
on the whole, remarkable   The most striking finding was that no case with
an impaired reaction gave a reaction within the normal range in any subse-
quent experiment, despite considerable changes in degree of bronchospasm
and bronchitis   The response of patients with a normal reaction to CO_2
was also consistent   The single exception was Case 20 who gave a markedly
impaired reaction in the second and third exposures   This patient had all
the characteristics of severe emphysema, and it was a surprising finding
when he gave a normal reaction in the first experiment   There was no
significant change in his condition over the period

Reaction to anoxia

In view of the possibility that the reactions of the respiratory centre
may be modified by adaptation to the abnormal internal environment in this
disease, the effect of anoxia was also investigated   The effect on ventilation
during the first five minutes of exposure to 12% O_2 was determined in ten
patients with emphysema, who were tolerant to CO_2, and eighteen normal
subjects   On the whole, the reactions of the two groups were very similar,
the usual wide range of response being found (Fig 7)   There were, however,
certain differences   Whereas the normal subjects showed the maximum
mean reaction in the second minute with a gradual falling off afterwards,
the emphysematous subjects reacted more slowly, the mean still rising at
the end of five minutes   This difference is just statistically significant
These results are difficult to interpret in further detail as the emphysematous

subjects were presumably in different ranges of anoxia to the normal subjects
It is impossible to assess the complicated interactions of peripheral stimula-
tion and central stimulation and depression   However, speaking in general
terms, this appears to be yet another example of the delay in physiological
response to changes in the respiratory environment, found in emphysema
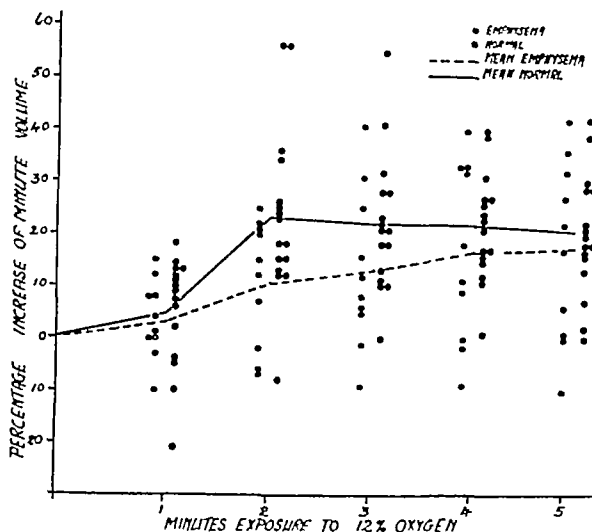
Fig 7   The response of ten CO_2 tolerant cases of emphysema and 10 normal subjects to 12%
oxygen in nitrogen   The mean values of the two groups are also plotted

Discussion

The observation that the reaction to CO_2 is impaired in emphysema
has been confirmed   The alkali reserve of all the patients with impaired
reactions was within the normal range (as established by Van Slyke) but
within this range there is considerable statistical correlation between the
respiratory response to CO_2 and the alkali reserve (Table I)   Although it
cannot be denied that increased buffering will reduce the stimulating action
of CO_2, the finding that the reaction is delayed rather than abolished suggests
that the main fault lies neither with the blood buffers nor with the sensitivity
of the respiratory centre   A delayed reaction of this type is not observed
when normal individuals are exposed to concentrations of less than 4% CO_2

A simple experiment was carried out in relation to this problem   Five
cases of emphysema, who had given impaired reactions to CO_2, breathed
oxygen for twelve minutes to ensure a high concentration of oxygen in the
lungs   They then held their breath for as long as possible   The period of
apnoea varied from 26 to 72 seconds, which is less than in normal subjects

following the same procedure It is hard to reconcile this finding with a true tolerance to carbon dioxide either by increased buffering or by adaptation of the respiratory centre

Mechanical limitation of the power to increase ventilation has also been excluded as a fundamental cause since many of the CO_2 tolerant cases had ample ability to ventilate voluntarily volumes that were well within the normal range of response to 4% CO_2 It has also been shown that there can be marked impairment of this reaction, with a normal increase in either tidal air or respiratory rate The fact that none of these patients complained of any discomfort would also suggest that immobility of the chest is not a causal factor

It is known that the ability to produce a homogenous mixture of gases in the lungs is impaired in emphysema (3) and the delayed response to CO_2 may be another manifestation of inefficient mixing of gases in the lungs It is difficult to believe, however, that defective mixing of gases could alone be responsible for the prolonged delay in the response to CO_2 which we have observed, and more direct evidence in favour of such an explanation would have to be produced before it can be accepted

The significance in emphysema of any diagnostic procedure is extremely difficult to establish since in this disease, the diagnosis can sometimes only be confirmed with certainty by a post-mortem examination The ordinary physical signs of emphysema, although present in most cases can be quite misleading (1) and the same is true of radiological examination and of the measurements of the lung volumes and its subdivisions (2) Any investigation is therefore unsatisfactory at the start since it is always possible that patients without emphysema may be included Tolerance to CO_2 is, however, unaffected by bronchospasm, and is normal or decreased in cardiac conditions uncomplicated by lung disease (6) This latter finding needs further investigation, but has been confirmed in a limited number of patients, although in two cases of emphysema with " cor pulmonale " increased tolerance to carbon dioxide was found

Summary

The reaction to 4% CO_2 of 28 normal, 23 emphysematous and 7 asthmatic subjects has been studied Of 23 emphysematous patients, 16 gave impaired reactions in the first five minutes of exposure The 7 asthmatic subjects gave normal reactions

The reaction of the CO_2 tolerant cases to anoxia and to longer exposures to 4% CO_2 has been investigated

The causes of the impaired reaction to CO_2 in emphysema and its diagnostic significance are discussed in relation to these findings

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REACTION TO CARBON DIOXIDE IN PNEUMOKONIOSIS OF COALMINERS

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ALTHOUGH pneumokoniosis has its own definite ætiology, natural history and radiological and clinical findings, it is nevertheless frequently associated with emphysema and the usual respiratory tests such as vital capacity, maximum breathing capacity, exercise tolerance and lung volume determinations are considerably affected by both diseases   In some cases it is impossible to state whether the emphysema is secondary or coincidental, or to what extent it is responsible for the respiratory disability   In patients with massive fibrosis the radiological appearance of emphysema may only be secondary to the hilar distortion and extreme scarring in the upper zones Gough (3) has also described a focal form of emphysema around the coal foci The part played by this disease process in the dyspnœa of pneumokoniosis is not yet determined   In view of this confused picture and the fact that an impairment of reaction to CO_2 has been demonstrated only in emphysema (Scott (4) , Donald and Christie (1)), the response of a number of patients with pneumokoniosis was investigated

Method

The reaction of 56 patients with pneumokoniosis to 4% CO_2 was studied, employing the technique and methods already described by Donald and Christie (1)   These patients were all under the care of the Pneumokoniosis Research Unit (M R C) and the large majority were certified cases who had been in-patients for a period of a month or more for assessment and rehabilitation   During this period they had been under careful observation and graded according to their degree of dyspnœa   Only one exposure to CO_2 was carried out on each subject and of 56 cases, 8 were rejected owing to unsatisfactory tracings while breathing air   It is of interest that four of these eight subjects who gave irregular respiratory tracings, usually with hyperventilation, were miners with definite pneumokoniosis but minimal dyspnœa   The history and clinical findings, X-ray report, vital capacity and maximum breathing capacity were obtained from the records of the Unit   The methods of determination of the vital capacity and maximum breathing capacity were those described by Gilson and Hugh-Jones (2)

TABLE I

The history, clinical and radiological findings, vital capacity and maximum breathing capacity and reaction to 4% carbon dioxide in 48 coalminers studied

Case No	Age	Yrs exposure	Yrs dyspnœa	Yrs certified silicotic	Bronchospasm	Blood pressure	Clinical	X ray	Vital capacity (c c)	Max breathing capacity (litres)	Max % increase minute vol with 4% CO_2*
							GRADE V — *Dyspnœic at rest*				
1	61	50	7	4	Pr	125/82	Wasting	—	1670	—	26
2	64	40	10	Not	0	110/80	Sputum+ Emph+	Emph++ N.D.D	1420	—	36
3	58	40	15	Not	+	190/100	Coronary disease	N.D.D	2860	2	40
4	60	40	7	3	Pr	110/60	Emph++ Clubbing Wasting+ Heart failure+	Emph+++ M S +	—	—	52 (2½)*
							GRADE IV — *Dyspnœic on slightest exertion*				
5	48	24	10	10	+	120/75	Emph++	M D D Emph+++	1667	25	30
6	60	46	17	2	0	120/80	Emph++	M.D.D Emph++	—	—	44
7	60	40	6	7	Pr	115/75	Emph Wasted Pleurisy	M S (Calc) Bas Emph	2950	—	40
8	53	32	10	5	+	140/85	Mod Emph	M S (Cav) Bas Emph	2700	24	49
9	56	33	14	10	Pr	115/80	Mod Emph	Nod Bas Emph	1940	26	53
10	45	35	8	5	0	130/75	Emph	Ret Bas Emph	1300	40	60
11	43	22	8	8	+	140/80	Wasted	M S+	2390	50	71
12	47	29	3	3	++	130/90	Wasted Heart+ Gallop L B B B	M S	1806	30	73
13	50	40	9	6	Pr	105/70	Asthma	Heart+ M S+	1740	25	82
14	51	29	9	9	+	115/70	Wasted	Nod Coal	2950	30	90
15	46	24	10	7	Sl	105/70	Pigeon chest Wasted	M S	2130	39	101

No											
16	54	30	11	12	0	120/70	Nil of note	Ret +	3080	67	23
17	62	38	10	Not	++	114/84	Emph ++	N D.D Emph ++	1910	33	32
18	53	14	8	7	++	90/65	Mod Emph Sputum+	M.S Bas Emph	2546	—	56
19	42	23	7	4	Pr	110/70	Wasted	Nod	2113	50	67
20	45	22	10	10	+	120/80	Nil of note	Coal Cav Nod Coal	1420	35	78
21	62	38	7	5	+	125/80	A C.D absent	M S Bas Emph	2920	32	84
22	52	28	2	Not	0	160/100	Nil of note	Nil of note	2320	43	85
23	51	30	4	Not	+	—	Mild emph	Ret	4150	71	94
24	51	29	9	9	Pr	115/70	Wasted	Ret Nod Coal	2950	30	90
25	39	16	12	9	0	180/100	Alb and casts Heart+	MS	2500	57	157 (24)

GRADE II *Dyspnoeic uphill or stairs*

No											
26	39	19	9	7	0	120/70	Nil of note	M S	2720	36	50
27	40	21	7	6	0	120/75	Nil of note	M S	3030	62	71
28	42	23	14	5	0	130/80	Diss Sclerosis	M S ? Emph	2990	43	71
29	45	27	9	5	0	110/80	Nil of note	M S (Calc)	2510	—	71
30	37	15	8	6	0	115/70	Rheumtd Arth Gallop	M S (Cav)	1610	52	90
31	43	25	6	5	0	150/75	Obese	Ret + Coal	4100	65	104
32	48	26	7	7	0	130/75	Nil of note	M S ? Bas Emph	3000	80	94
33	50	28	7	9	0	140/85	Nil of note	Ret	4250	134	123
34	44	27	3	5	0	110/70	Nil of note	Ret	2910	50	71
35	42	23	—	6	0	140/85	Nil of note	Ret + Coal	3040	81	74
36	40	15	6	6	0	110/60	Obese	M S	2790	90	87
37	53	32	4	Not	0	140/85	Neurasthenia	Ret	3000	57	87

TABLE I—*continued*

Case No	Age	Yrs exposure	Yrs dyspnoea	Yrs certified silicotic	Bronchospasm	Blood pressure	Clinical	X ray	Vital capacity (c c)	Max breathing capacity (litres)	Max % increase minute vol with 4% CO₂.
							GRADE II *Dyspnoeic uphill or stairs—continued*				
38	45	15	10	15	Sl	120/80	Sl Emph Liver? F B +	M S Emph Bns	—	—	88
39	44	26	6	4	Sl	130/80	Nil of note	M S	3340	45	94
40	46	25	7	5	0	125/80	Wasted Rheumtd Arth	Nod Coal	2770	64	97
41	38	19	7	6	0	140/90	Nil of note	M S	2080	64	132 (4)
42	33	18	3	Not	0		Still at face	Ret +	3010	103	110
43	42	19	10	9	++	118/90	Nil of note	Nod Coal	—	—	120
44	45	30	8	4	++	130/90	Thyrotoxicosis Sputum +	Ret + Coal Cav	3750	71	120
							GRADE I *Dyspnoeic maximal exertion only*				
45	35	17	4	4	0	120/70	Still labouring	Ret	3060	142	78
46	34	20	—	1	0	140/80	Still mining	Nod Coal Early Emph	4650	100	60 (3)
47	35	10	—	5	0	135/90	Still labouring	Ret +	3770	135	85
48	32	13	—	6	0	120/75	Still labouring	Ret +	4083	86	94 (4)

Key to Table I

1	N D D	No dust deposits	7	M S	Massive shadows
2	M D D	Minimal dust deposits	8	Cav	Cavitation
3	Ret	Reticulation	9	Calc	Calcification
4	Ret +	Marked reticulation	10	Emph	Emphysema
5	Nod	Nodulation	11	Bas	Basal
6	Coal	Coalescence	12	Pr	Present

* *Reaction to CO₂.* If exposure shorter than 5 minutes, time is stated in brackets in minutes

Results

The results are shown in Table I   The patients were divided into five groups according to their degree of dyspnœa   The age, period of exposure to coal dust, duration of dyspnœa, degree of spasm, clinical findings, X-ray report, vital capacity, maximum breathing capacity and number of years certified are given   The findings are further summarised in Table II

In Group V (dyspnœa at rest) only four patients were studied   Three showed marked impairment of reaction to CO_2, and two of these (Case 2 and 3) although both miners with a forty years history of exposure, had not been certified as there was no radiological evidence of pneumokoniosis but only of severe emphysema   The third had only moderate signs of emphysema   The fourth case was in severe degenerative heart failure   His X-ray showed massive shadows of pneumokoniosis   The minute volume while breathing air was over 10 litres and although he responded normally to 4% CO_2 (52% increase after 2½ minutes), he was unable to tolerate any further exposure

In Group IV (dyspnœa on the slightest exertion) six out of eleven subjects showed impairment of reaction to 4% CO_2 The two most tolerant cases had, radiologically, minimal dust disease but severe emphysema   Of the other four tolerant cases two had massive shadows, one nodulation and one reticulation   Marked emphysema of the bases was noted in the X-ray report of three of these patients and generalised emphysema in the patient with reticulation   It is emphasised that these reports were made by an independent observer some months before these experiments were initiated   Of the five subjects giving normal reactions to CO_2, four had massive shadows and one coalescence of nodules   One of these cases (12) had bundle branch block with an enlarged heart and gallop rhythm   Another point of interest was that two of the tolerant subjects had vital capacities well above the mean of the normal reactors in this group

In the third group (Grade III—dyspnœa walking at moderate speed on the level) only three out of ten subjects showed impaired reactions to 4% CO_2 The most tolerant subject had only moderate signs of emphysema and had diffuse reticulation   The second tolerant subject had signs of gross emphysema and his X-ray report stated   " Severe emphysema with bullæ   Minimal dust deposits "   Although he had a thirty year history of exposure he had not been certified as a pneumokoniotic   The third tolerant subject had massive shadows and considerable emphysema at the bases   Of the normal reactors two had massive shadows, three coalescent nodules (one with cavitation), one reticulation only, and one no definite abnormality   In the X-ray reports of these cases the only mention of emphysema was in Case 21 where it was stated " emphysema developing at left base "   Case 25, who had massive shadows, gave a very brisk reaction to 4% CO_2 and was so distressed after 2½ minutes that the experiment was stopped   Because of this his urine was examined and severe albuminuria and many casts were found   He also had hypertension

The next group (Grade II—dyspnœa climbing hills but capable of moderately heavy exertion) contained 19 subjects and of these only one patient showed increased tolerance to CO_2 He had massive shadows but no clinical evidence of emphysema, although there was a ten year history of bronchitis   His vital capacity was 2,720 c c and his maximum increase of minute volume in the first five minutes was 50%   The other eighteen subjects had reactions well within the normal range   Seven had massive shadows and the remainder varied from nodulation with coalescence to minimal reticulation   One subject, a severe asthmatic, who was tested while in severe bronchospasm, increased his minute volume by 120%

The group with minimal dyspnœa (Grade I) contained 4 subjects These men were capable of fairly heavy labour   All had been certified , some were still miners and others were working at labouring jobs   All gave normal reactions to CO_2 Two of these had nodulation with coalescence and in one subject the X-ray suggested some degree of generalised emphysema although his vital capacity was 4,550 c c   It will be seen that the maximum breathing capacity and vital capacity of these subjects was normal or near normal

Discussion

A number of the more markedly impaired reactions to carbon dioxide were found in miners with little or no pneumokoniosis but with severe emphysema (Cases 2, 3, 5, 6, 17)   Three of these patients had not been certified as pneumokoniotics although they were severely dyspnœic and had worked underground for 38 to 40 years (Cases 2, 3, 17)   Eleven of the thirteen subjects who gave impaired reactions to carbon dioxide had marked clinical and radiological evidence of emphysema   Only six out of the thirty-four pneumokoniotic patients with a normal reaction had any clinical or radiological evidence of emphysema, and in none of these cases was this evidence marked   Patients with pneumokoniosis complicated by heart failure, nephritis and neurasthenia all gave normal reactions

These findings, in a disease where pulmonary fibrosis and emphysema so frequently co-exist, are further evidence that an impaired reaction to carbon dioxide is only obtained if true emphysema is present   Although the incidence of emphysema, with impaired reaction to carbon dioxide, increases in those severely disabled by pneumokoniosis it must be emphasized that a considerable number of miners were extremely dyspnœic with no signs of emphysema and a brisk reaction to carbon dioxide (Cases 11, 13, 14, 15, 19, 20, 21, 23, 24)   If one accepts carbon dioxide tolerance as a specific test for emphysema these results would indicate that, in many cases of pneumokoniosis, the dyspnœa is not due to emphysema

TABLE II

Degree of clinical emphysema, radiological emphysema, pneumokoniosis and reaction to carbon dioxide in 48 coalminers

Grade of dyspnoea	5	5	5	5	5	4	4	4	4	4	4	4	4	4	4
Case No	1	2	3	4*	5	6	7	8	9	10	11	12*	13	14	15
Emphysema clinically	++	++	++	++	++	++	+	+	+	+	0	0	0	0	++
Emphysema radiologically	—	++	++	++	++	++	+	+	+	+	0	0	0	0	+
Pneumokoniosis	Pr	0	0	++	Sl	Sl	0	++	+	+	++	++	++	+	++
Impaired CO₂ tolerance	+	+	+	N	+	+	N	+	+	+	N	N	N	N	N

Grade of dyspnoea	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2
Case No	16	17	18	19	20	21	22	23	24	25*	26	27	28*	29	30*
Emphysema clinically	0	++	+	+	0	Sl	0	Sl	0	0	0	0	0	0	0
Emphysema radiologically	0	++	+	+	0	Sl	Sl	0	0	0	0	0	0	0	0
Pneumokoniosis	+	0	0	+	++	++	++	++	+	++	++	++	++	+	++
Impaired CO₂ tolerance	++	+	+	N	N	N	N	N	N	N	+	N	N	N	N

Grade of dyspnoea	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1
Case No	31	32	33	34	35	36	37*	38	39	40*	41	42	43	44*	45	46	47	48
Emphysema clinically	0	0	0	0	0	0	0	Sl	0	0	0	0	0	0	0	0	0	0
Emphysema radiologically	0	Sl	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	Sl
Pneumokoniosis	+	++	Sl	+	+	++	Sl	+	++	++	+	++	+	+	Sl	+	+	+
Impaired CO₂ tolerance	N	N	N	N	N	N	N	N	N	N	N	N	+	N	Sl	N	N	N, N

Emphysema radiologically, Emphysema clinically   ++ = gross   + = definite   Sl = slight

Pneumokoniosis   ++ = massive shadows   + = coalescence, nodulation, marked reticulation
Sl = minimal reticulation, minimal dust deposits   Pr = Pneumokoniosis present   Certified but X ray not seen   * = complicated (*see* Table I)

Impaired CO₂ tolerance   + = sub normal reaction   N = Normal reaction

The disability of miners without emphysema (as judged clinically, radiologically, and by their reaction to carbon dioxide) appears to be little related to the severity of the pneumokoniosis and this would suggest that some other factor, which cannot be appreciated by radiological examination, is involved

SUMMARY

The reaction to 4% carbon dioxide of a group of miners with pneumokoniosis has been determined   It was found that only those cases showing dyspnœa with other evidence of emphysema gave impaired reactions   A number of subjects with marked dyspnœa and pneumokoniosis gave normal reactions   The significance of these findings is discussed

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ŒDEMA AND POTASSIUM LOSS
IN COMBINED SODIUM p-AMINOHIPPURATE AND PENICILLIN THERAPY

A METABOLIC STUDY

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PENICILLIN is rapidly excreted by the kidneys, mainly by the tubules which eliminate about four times as much as the glomeruli (20)   The excretion of penicillin is delayed when diodrast (diodone), which is also excreted by the tubules, is given at the same time (19)   Beyer and others (2, 3) showed that in dogs and man intravenous infusions of sodium p-aminohippurate increased the plasma levels of penicillin in the same way as diodrast, large doses caused œdema but no other toxic signs in dogs   Loewe and others (14) observed that in short clinical trials sodium p-aminohippurate stimulated smooth muscle but concluded that it was not otherwise toxic

With ample supplies of penicillin there is no need to interfere with tubular function except in rare cases when massive doses fail to control an infection   Loewe and others (15) treated a patient, who had subacute bacterial endocarditis due to a resistant organism, with 10 million units of penicillin and 240 g of sodium p-aminohippurate daily for thirteen days   The patient was cured and no toxic effects were reported   This paper records toxic effects of a similar course of treatment and describes disturbances of electrolyte metabolism induced by sodium p-aminohippurate

The patient, a man aged 63, was admitted in October, 1946, with a five months' history of subacute bacterial endocarditis affecting the aortic valve   There was transient œdema of the ankles but no other sign of heart failure   Blood culture showed a slowly growing *streptococcus viridans* which was inhibited by 2 units of penicillin per ml   Increasing doses of penicillin failed to cure the infection and by January, 1947, the patient had received 10 million units of penicillin daily for two months   The organism became more resistant so that it grew in 6 units and was inhibited in 8 units per ml

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Therapeutic course of penicillin and sodium p-aminohippurate   While a supply of sodium p-aminohippurate was being prepared,* Loewe's report (15) came to hand, so the drug was given in the same way   From February the 17th, a 12% solution of sodium p-aminohippurate, adjusted to pH 6 8 to 7, was given continuously using a Jouvelet transfusion machine At first about 240 g of the sodium salt were given each day, this gave serum levels of 68 to 74 mg per 100 ml   The dose was reduced to 160 g each day, and was later altered slightly so as to maintain a serum level of about 40 mg per 100 ml   Each day 10 million units of penicillin and 50 mg of heparin were given in the infusion

Effects of therapy   The patient complained of thirst and drank two to three litres of water each day   He lost his appetite, and after five days became lethargic, weak and drowsy, he would doze and let fall a thermometer within a minute of its being put in his mouth   Breathing became shallow and he was slightly cyanosed, the cyanosis was deeper while he was asleep and was abolished promptly when he was asked to take deep breaths Although his muscles were very weak, and his jaw and lips drooped, he had no true paralysis

From the second day he retained fluid   Œdema appeared after four days and steadily increased, involving the buttocks, back, abdominal wall and finally the conjunctivæ   Pleural effusions and ascites developed after the sixth day and the circumference of the belly increased from 32 to 37 inches   The lungs were clear, but the patient was kept propped up to avoid pulmonary œdema   The venous pressure did not rise and the liver did not enlarge

Because the patient had become more cyanosed and ominously drowsy, the p-aminohippurate drip was stopped on the eleventh day, February the 28th, but the patient's weakness and œdema improved only slowly Improvement was quicker when potassium chloride was given   Penicillin was continued, the dose being raised to 40 million units a day, and was given intramuscularly every three hours for twenty-two days   Six days later a blood culture was positive once more

The striking finding in the blood was a low serum potassium   On three occasions when it was estimated during the infusion it was 9 2, 10 and 11 mg, and it remained between 10 and 12 mg until two weeks after the end of the infusion when 3 g of potassium chloride were given daily, when it rose to between 16 and 18 mg per 100 ml   The serum chloride fell, and during the last few days of the infusion the CO_2 combining power rose from 73 to 96 volumes per 100 ml

The levels of penicillin in serum were estimated by serial dilution, using serum as diluent   When 10 million units of penicillin were given daily with sodium p-aminohippurate the average of seven estimations was 67

* By Ward, Blenkinsop and Co

units per ml   Comparable levels were found when each day 40 million units were given alone   When 10 million units were later infused alone, the average of five estimations was 11 units per ml

Clearly sodium *p*-aminohippurate increased the plasma levels of penicillin   It was therefore thought that a longer combined course should be tried if the clinical and chemical upsets could be prevented   But the cause of the upsets was not known, so a short course of sodium *p*-amino-hippurate was given and the effects of this on sodium and potassium metabolism were studied

This metabolic study threw some light on the disturbances of electrolyte metabolism, but a further long course of sodium *p*-aminohippurate and penicillin was not given because a supply of caronamide was received Penicillin was given for several months (for two with caronamide), and, although the infection recurred, the patient became fit enough to walk about slowly   He died in December, 1947 , autopsy revealed large, partly healed and calcified vegetations on the aortic valves with recent extensions on to the mitral

The metabolic study

As 240 g of sodium *p*-aminohippurate contains 25 5 g of sodium it seemed probable that much of the toxic effects could have been due to retention of some of this sodium   But the fall in serum potassium could not be explained   Therefore the metabolic study was designed both to cover the balances of sodium and potassium, and, by adopting Darrow's method (7) of following the chloride, nitrogen and body weight, to deduce shifts of sodium and potassium between the intracellular and extracellular fluids

Methods   Streptomycin (4 g a day) was begun on May 1st and continued for a month   The metabolic study began at 10 a m on May 4th, and covered ten days   There were four successive periods

1   Two days (1 and 2) while the patient became used to the diet

2   Five days (3, 4, 5, 6 and 7) covering the infusion and excretion of sodium *p*-aminohippurate   The infusion was given for 99 hours—in the following 21 hours most of the *p*-aminohippuric acid left the body   1520 ml of a 12% solution was given each day   The total of sodium *p*-aminohippurate was 755 g , 80 g of which were sodium

3   One day (8) without treatment

4   Two days (9 and 10) on each of which the patient was given 10 g of potassium chloride by mouth
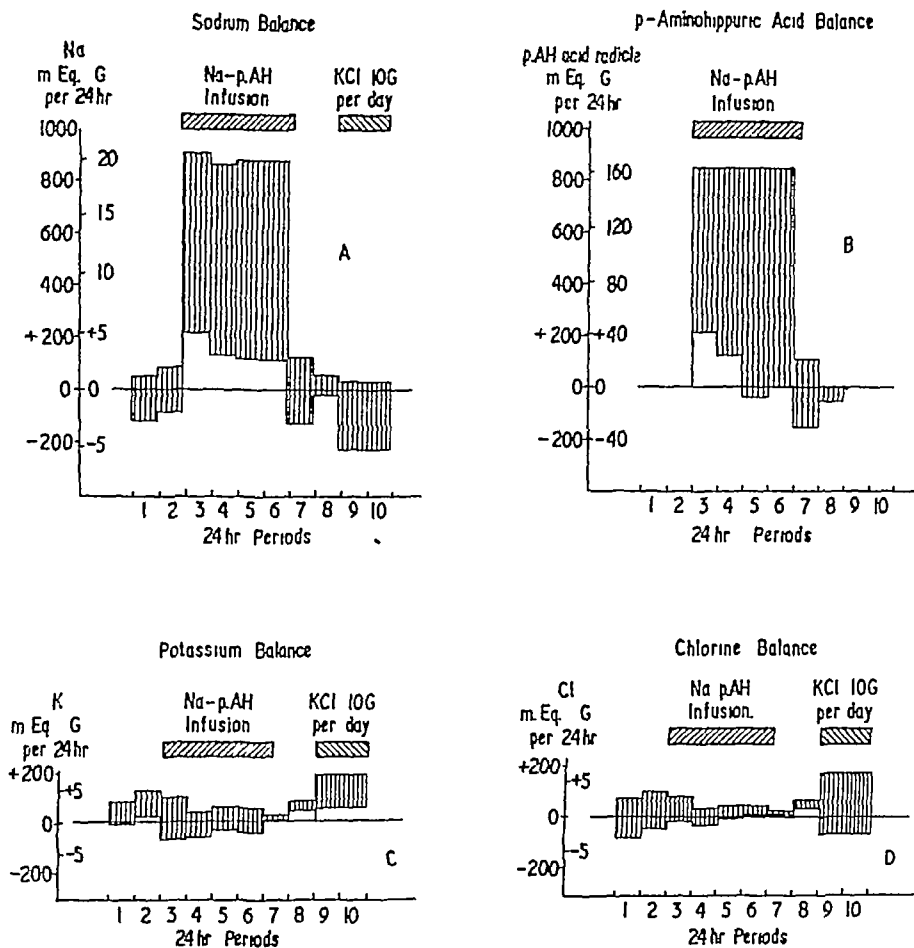
Fig 1 Ordinates are calibrated in grams and milli-equivalents, the scale of the latter is constant Intakes are shown as distances between the upper and zero lines, outputs are the lengths of the shaded columns An unshaded column between the output and zero line represents a positive balance (retention), a shaded column below the zero line represents a negative balance (loss) The abscissæ are calibrated to show periods of 24 hours, from 10 a.m to 10 a.m

Balance data are in Table I

The diet consisted of milk, eggs, glucose and water   The composition of milk was assumed to be that quoted by Cox and Mueller (6)   Charcoal and carmine were used alternately in an attempt to mark the stools   The urine was preserved with thymol

Analytical methods   Potassium in serum urine and fæces—Jacobs and Hoffman's method   Sodium in serum urine and fæces—Butler and Tuthill's application of Barber and Kolthoff's method   p aminohippuric acid—Bratton and Marshall's method modified by Goldring and Chasis (11)   Nitrogen in urine—Kjeldahl's method

Ashings were done at 380 to 400°C for six hours   Many analyses were repeated more than once and were done in parallel with known solutions   The results agreed to within 5 per cent

Sodium found in the p aminohippurate infusion was within 2 per cent of the theoretical value   The infusion contained no potassium or barium   The results of other investigations including hæmatocrit, red cell potassium and urinary urea were not of value and are not shown

The day to day outputs have a small error because the patient could not pass his urine promptly   Some of the specimens of urine passed during the last two days of the study were pooled before being analysed, so the average balances are shown   The stool from days 9 and 10 was lost, but the error involved is probably trivial (13)

Results

Clinical effects   The patient found the diet uninteresting   The infusion made him thirsty , he lost his appetite and became lethargic   He complained of some weakness, but his grip gave the same readings on a dynamometer   There was no œdema and, as in the first few days of the previous course, his general condition was good   The vein thrombosed on the last day and some of the infused fluid escaped into the tissue

Electrocardiograph changes   Depression of T waves appeared in leads II and III after the infusion had been given for 24 hours, and persisted until potassium chloride was given   These changes appeared before the serum potassium fell (24)

Chemical findings   The balance data are given in Table I   The intake, output and daily balance of sodium, potassium, chlorine and the p-aminohippurate radicle are depicted in Fig 1 A to D , the scales for both milliequivalents and grams are shown   The values of the blood analyses are shown in Table II, the time and date of collection is shown to avoid ambiguity   The concentrations in the urine of sodium, potassium, chlorine and the p-aminohippurate radicle are shown in Table III   Fig 2 shows the cumulative balances of nitrogen and potassium , the scales are related to the relative amounts of each found in body protein

Significance of findings

A 12% solution of sodium p-aminohippurate is 555 m mol per litre, so the concentration of sodium in it is about four times that in plasma   Sodium was retained on each of the four full days of the infusion, though the retention was greatest on the first day   The concentration of sodium in the urine rose parabolically, levelling off at 273 m eq per litre   The plasma level rose throughout   In the two days following the infusion sodium was lost at the same rate as p-aminohippurate   But it was not until potassium chloride was given that most of the sodium previously retained was lost

CATES

TABLE I

Balance data

From 10 a m, May 4th, to 10 a m, May 14th

Intake	Unit	Day								9 and 10 Total
		1	2	3	4	5	6	7	8	
Water, by mouth	ml	2225	2818	3130	2579	3307	2331	1892	2895	4130
,, by drip	ml	—	—	1520	1520	1520	1520	190	—	—
p aminohippuric acid radicle	m eq	—	—	843	843	843	843	106	—	—
Sodium in drip	m eq	—	—	843	843	843	843	106	—	—
,, in diet	m eq	56	85	65	23	37	36	15	53	67
Potassium in diet	m eq	87	127	98	35	50	54	24	80	101
,, in KCl	m eq	—	—	—	—	—	—	—	—	208
Chlorine in diet	m eq	70	101	70	29	46 5	44	20	66	83
,, in KCl	m eq	—	—	—	—	—	—	—	—	208
Nitrogen in diet	g	15 2	20 9	17 8	5 06	0 8	10 06	4 48	13 1	17 2
Calories		1830	2613	2035	999	1192	1258	630	1523	2510

Output

Urine:										
Volume	ml	1900	2050	3560	3060	2020	2700	900	2070	4870
p aminohippuric acid radicle	m eq	—	—	637	722	884	838	250	53	—
Sodium	m eq	170	165	682	726	756	758	247	72	518
Potassium	m eq	82	92	100	92	87	94	10	37	268
Chlorine	m eq	155	143	91	61	46	34	9	29	497
Nitrogen*	g	12 45	15 8	22 32	15 0	9 36	16 1	4 54	11 2	14 7
Faeces										
Sodium	m eq	5	5	7	7	7	4	4	4	?
Potassium	m.eq	9	9	2	2	2	2	3	3	?
Chlorine	m eq	2	2	4	4	4	4	2	2	?
Nitrogen (allowed)	g	1 25	1 25	1 25	1 25	1 25	1 25	1 25	1 25	2 5
Weight	kg	57 7 a m	56 8 p m					57 3		54 1

* Excluding nitrogen in p-aminohippuric acid radicle

During the infusion the weight gained was only 0 5 kg   Indirect estimations of the change in extracellular volume by the methods of Darrow (7) and of Reifenstein (21) suggest an increase of 1½ to 2 litres, of which a half may have come from the intracellular fluid   The amount of sodium retained was more than could be accounted for by the rise of serum sodium and by the calculated increase in extracellular volume   This suggests that
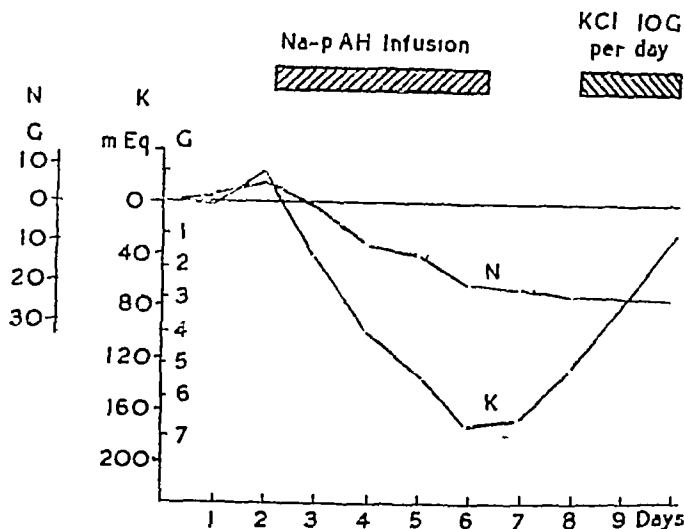
Fig 2   Cumulative balances of nitrogen and potassium related to one another in the proportions they are found in protoplasm   (1 g of nitrogen — 3 m eq (117 mg) of potassium)
Infusion of sodium *p* aminohippurate induced a loss of potassium greater than that attributable to breakdown of protoplasm
After the infusion had been stopped potassium was stored in the body, although there was no retention of nitrogen

some sodium entered the cells   When potassium chloride was given the weight lost was 3 kg   The same estimations indicate that 3 litres of water from the extra-cellular fluid were lost in the urine   All the sodium lost during this time seems to have come from the extracellular fluid

Potassium was lost on each day of the infusion   The serum level fell throughout   There was also a loss of nitrogen, indicating destruction of body protein   The amount of potassium lost was more than could be attributed to the destruction of body protein and the loss of potassium from the extracellular fluid together   After the infusion potassium was retained, both spontaneously and when potassium chloride was given   The amount retained was more than that gained by the extracellular fluid   No nitrogen was retained, which suggests that the retained potassium was not used in rebuilding protein

At first the *p*-aminohippurate radicle was retained, but the daily output in the urine equalled the intake after the second day   Thereafter equilibrium was maintained until the infusion was stopped, when the retained amount

was excreted mostly in the first and the rest in the second day   The plasma level of the p-aminohippurate suggests that the radicle had fully entered all the body water by the end of the second day of the infusion   The large amount excreted after the infusion supports this   The recovery of the p-aminohippurate was 97 per cent

DISCUSSION

In general, when sodium salts are infused water and sodium are retained (26), and there is no reason to suspect any other mechanism caused the œdema and alkalosis in this patient   He was afterwards able to walk about slowly without congestive failure, so heart failure cannot be blamed   And he had further large doses of penicillin without ill effect

Loss of potassium from the body and sometimes a fall in serum potassium may follow dehydration (4) or infusions of hypertonic (8) and even isotonic solutions (10, 23)   When hypertonic infusions are given to dogs potassium passes from the body cells into the extracellular fluid, but only when it is being excreted by the kidneys (9)   This renal mechanism may have caused some of the loss of potassium and the fall in serum potassium in this patient

In a variety of conditions a fall in serum potassium has been shown to cause paralysis, which is sometimes fatal, and other ill effects (1, 5, 10, 12, 16, 17, 25)   So the severe muscle weakness during the first infusion of sodium p-aminohippurate may have been due to the low serum potassium

When potassium salts are given to man there is a loss of water from the body   This occurred in the metabolic study and in Wolf's experiments on normal men (26) ,   it may happen in patients with œdema (18)   A low potassium intake, on the other hand, causes ascites in rats (22)   The behaviour of the œdema after the first infusion of sodium p-aminohippurate suggests that the loss of potassium during this infusion was a factor in maintaining the œdema

The amount of sodium given to this patient was very large ,   in the initial daily dose of 240 g of sodium p-aminohippurate there were 25 5 g , the same as in 65 g of common salt   The danger of sodium retention is well known, and the clinical effects of giving the long course of sodium p-aminohippurate illustrate this danger   But the findings in the metabolic study also suggest that the clinical effects of too much sodium may be in part due to secondary disturbances of other electrolytes of which potassium deficiency seems to be one

SUMMARY

Sodium p-aminohippurate was used to enhance levels of penicillin in the treatment of bacterial endocarditis   This caused œdema, muscular weakness, alkalosis, and a low serum potassium

TABLE II
Blood analyses
(milli equivalents per litre)

Substance	5th	6th		7th	8th	9th	10th	12th	14th
Date / Time	noon	8 45 a m.	3 p m	11 a m	10 a m	10 a m	8 p m	3 p m	noon
					May				
Sodium in serum	137	—	—	—	143	145	147	149	151
Potassium in serum	5 2	5 3	4 9	4 3	4 1	—	3 3	3 9	5 4
Alkali reserve	25 1	27 3	22 5	25 1	30 2	28 3	—	35 5	28 5
Chlorides in serum	95 4	96 9	99 1	91 9	90 9	84	91 9	86	97
p aminohippuric acid	—	—	1 2	2 03	2 01	—	—	—	—

TABLE III
Concentrations of substances in urine
(milli equivalents per litre)

Substance	1	2	3			4	5	6	7	8	9	10*
			1 hr	5 hr	24 hr					1 18 hr		
					Day							
p aminohippuric acid (mol wt 194)	—	—	51	101	200	236	303	300	288	35	—	—
Sodium	90	81	106	151	215	238	259	272	273	35	99	127
Potassium	43 6	45 1	60 2	43 1	43 6	30	20 7	33 8	21	18	51 3	03 8
Chlorine	82	70	76	30	18	20	16	12	10	14	86	144

* Last specimen of the day

In a subsequent study of the effects of sodium p-aminohippurate on metabolism it was found that the body retained sodium and lost potassium The significance of these findings is briefly discussed

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THE EFFECT OF TRAUMA ON THE CHEMICAL COMPOSITION
OF THE BLOOD AND TISSUES OF MAN [*]

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THE BRITISH TRAUMATIC SHOCK TEAM II, R A M C (1945-6)

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DURING the recent war laboratory studies had directed our attention to the possibility that nucleotides might play a part as "toxic" metabolic factors in the response of the body to injury This arose from the demonstration that the shock-inducing factor in extracts of voluntary muscle is adenosine triphosphate (3, 16) We have also found that, in animals, trauma alters the distribution of nucleotide derivatives in the blood and tissues (4 17, 37, 38, 40) As experimental observations had shown (39) that a similar change could be detected after experimental limb ischaemia in man, it seemed important to study the effect of trauma on the distribution of nucleotide derivatives in the blood of man

An opportunity to do so came with the invasion of N W Europe in the summer of 1944 and after a preliminary investigation of the conditions and available facilities in forward areas it was decided to study clinical material on a large scale A team was formed and equipped with a mobile laboratory and other transport so that it would be sufficiently mobile and independent to move amongst the medical units at Corps level according to the flow of casualties The formation of this unit with the training of its R A M C personnel led to some delay between the initial observations in September

and October, 1944, and the full scale operations in the following year   The main series of our observations were made at Casualty Clearing Stations and Forward General Hospitals (250 beds) during the Battle of the Rhine and afterwards until the cessation of hostilities   The nature of the problem made it impossible to carry out all observations on the spot   Thus the determination of the adenosine equivalent was by biological assay and necessarily undertaken in our home laboratory

Altogether we were able to obtain material for chemical examination from 177 cases of battle injury   Before these patients reached us they had usually received first aid treatment at the Regimental Aid Post and Field Ambulance   Most of them were given morphine at this stage in doses up to $\frac{1}{3}$ gr and some were transfused   However, in all but four, observations
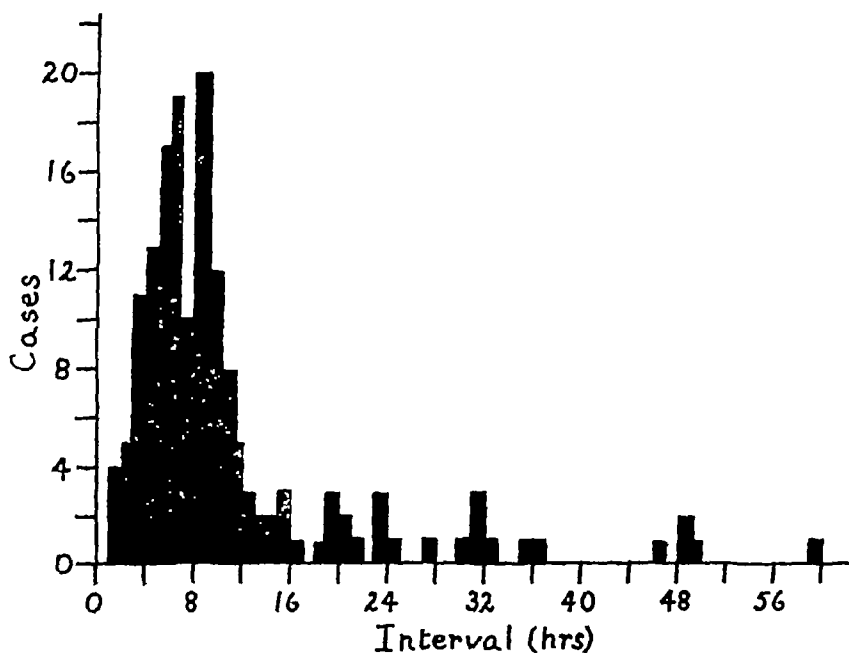
Fig 1   Frequency distribution of intervals between wounding and examination

were begun before surgical operation and in 128 of the total of 177, before transfusion   By the time the cases reached us they had been wounded for about 10 hours   The range of the intervals between wounding and examination is shown in Fig 1   Whilst head and chest injuries were excluded as far as possible we made no further attempt to discriminate between the type of case chosen for study   Routine clinical observations were made in order to classify the cases accurately, adhering as far as possible to the scheme suggested by Grant (14, 15)   Grant's full classification of

TABLE I

Distribution of cases according to diagnosis and number of deaths in each group during the first week after injury

| No of controls | Total No of cases | Muscle and bone injuries | | | Abdominal and thoraco abdominal injuries | Burns | Fat embolism | Gas gangrene |
		Slight Under ½ fistfuls of damaged tissue	Moderate ½ to 2	Severe 2 or over				
101	177	22	76	47	22	6	5	2
No of deaths	10	3	5	7	2	0	3	0

TABLE II

Distribution of cases according to degree of haemorrhage and initial blood pressure When the initial blood pressure was less than 100 mm Hg the cases have been further subdivided according to their initial pulse rates

| | Haemorrhage | | | Blood pressure | | | Pulse rate beats/min | | |
	Slight	Moderate	Severe	140 mm Hg and over	101 130 mm Hg	100 mm Hg and below	Under 70	70 100	100 and over
No of cases	74	72	23	34	65	56	1	13	41
No of deaths	3	6	7	2	2	11	1	2	8

injuries had to be abbreviated slightly because of the relatively small number of cases   Special care was taken to be present at operation as this was often the only occasion on which the amount of damaged tissue could be estimated The subdivision of our cases according to the type of injury is shown in Table I, according to the amount of hæmorrhage, and to their initial blood pressure and pulse rate in Table II   The criteria of blood loss were that less than ½ pint was considered slight, over 2 pints severe and intermediate amounts moderate   Only cases with an initial blood pressure of less than 100 mm Hg were subdivided according to their initial pulse rates   These tables also show the number of deaths in each group   More deaths may have occurred after the cases had passed out of our hands but we had no means of tracing them   Although we followed up the cases as long as possible, we were often prevented by military reasons from observing them for more than 48 hours   The figures given probably represent the mortality during the first week after injury

Clinical methods

(1)   *Blood*   As soon as possible after admission each patient was bled from a vein through a large bore transfusion needle without the aid of a syringe   Two 15 ml centrifuge tubes, containing 0 2 ml sat Na citrate, were filled with blood and a further 1 ml collected in a small test tube in which 0 1 ml Wintrobe's fluid had been dried   The following procedures were carried out in the mobile laboratory as quickly as possible

(a)   *On the blood in the small test tube* —This specimen was used to determine the hæmoglobin (Sahli), the hæmatocrit (centrifugation) and the blood sugar (micro Folin-Wu method)

(b)   *On the blood in the centrifuge tubes* —1 0 ml blood was added to 1 5 ml 10 per cent trichloroacetic acid and extraction of the adenosine-like substances begun according to the method of Barsoum and Gaddum (1) 2 0 ml were taken for an NPN determination (Folin-Wu)   The remainder was then centrifuged for 7 min at 3000 r p m when the plasma was separated and distributed as follows —4 0 ml were added to 6 0 ml 5 per cent trichloroacetic acid and filtered through Whatman No 42 paper after standing for 1 hour   4 0 ml were used to determine the inorganic and " 7 min " P by Brigg's method   1 0 ml was used to determine the amino-N content by Danielson's method (10)

The Barsoum and Gaddum extracts of whole blood and the trichloro-acetic acid extracts of plasma, together with certain muscle extracts to be described, were collected in the refrigerator until they could be transferred to the laboratory in Sheffield   They were flown from the continent to

Bristol and the journey from Bristol to Sheffield completed by train   The specimens were packed in insulated boxes with ice inserts and by changing these at Brussels and Bristol it was possible to keep them cool throughout the journey   On arrival in Sheffield they were stored at 4°C

In Sheffield the analyses were completed   The adenosine equivalent of the whole blood extracts and of neutralized aliquots of the trichloroacetic acid extracts of the plasma was determined by the guinea-pig atrium technique (11) as modified by us (37) and the results for the whole blood extracts corrected for variations in the Hb level as described there   Owing to the large number of specimens and the small amount of material available for tests it was not possible to do the assays in duplicate   Pentose determinations were also made on the trichloroacetic acid extracts of the plasma by Dr M Bielschowsky to whom we are indebted for these results and who used a modification of Mejbaum's (33) method   The full details of the method will be published by her elsewhere, but may be obtained on reference to the Medical Research Council (17)   The method estimates the phosphorylated fraction of the total pentose, that is, that part of the pentose which is present either as ribose phosphate or as nucleotide   This fraction is referred to in the text as Pentose P   Pentose A and B both refer to the total pentose, A before and B after dilution of the specimen in order to overcome glucose interference

In some cases it was convenient to prepare acetone precipitates of the plasma   The volume of the plasma obtained was measured and added to 7 vols of acetone   The specimen was then transferred to the Sheffield laboratory without refrigeration   The precipitates were filtered off, washed with acetone and ether, and dried in air at room temperature   After grinding to a fine powder the precipitates were weighed and 0 9 per cent NaCl extracts made corresponding to the original plasma volume   The adenosine equivalent of the plasma was determined on this extract as before   Trichloroacetic acid extracts (4 vols 10 per cent) were made from the saline extracts and the inorganic and " 7 min " P and the pentose fractions determined as above

A control series of blood samples was obtained from 164 soldiers who had recovered from minor injuries and illnesses and who were about to be discharged to their units   The specimens taken from these men were treated in exactly the same way as those taken from the battle casualties

2   *Muscle*   When possible, specimens of damaged muscle were obtained at the time of operation   Control specimens of apparently normal muscle were obtained from the wound area at the same time   These specimens were quickly taken to the mobile laboratory, where they were weighed and ground up in a mortar with quartz and 5 per cent trichloro-acetic acid (10 ml per g wet wt)   Extraction continued overnight in the

TABLE III

Mean values for haemoglobin, haematocrit, blood sugar, non Protein nitrogen and amino nitrogen levels for the different groups of cases
The criteria for the classification of the cases are as for Tables I and II

		Hb (Sahli)	Ht	Blood sugar mg per cent	N P N mg per cent	Amino N mg per cent
Muscle and bone injuries	Slight	86	42	137 0	50 1	3 65
	Moderate	79	39	152 0	48 4	3 25
	Severe	69	34	108 0	43 7	3 06
Abdominal and thoraco abdominal injuries		84	40	108 5	49 9	3 16
Burns		96	43	100 0	41 4	—
Haemorrhage	Slight	85	43	148 0	47 6	3 55
	Moderate	73	35	151 0	49 8	2 97
	Severe	73	36	177 0	43 2	3 17
Blood pressure	140 mm Hg and over	83	41	123 0	47 3	3 38
	101 139 mm Hg	80	40	152 4	48 0	3 32
	100 mm Hg and below	77	36	170 0	47 8	3 09
Pulse rate beats per min	Under 70	80	35	131 0	50 7	2 79
	70 100	83	43	143 0	45 8	3 36
	100 and over	74	34	172 6	48 2	3 02
Controls		102 5	46	98 3	—	3 3

TABLE IV

Mean values for the blood levels of the various components of the "nucleotide complex" in the different groups of cases. The criteria for the division of the cases are the same as in Tables I and II. The control mean values are shown for comparison.

		Inorganic phosphate mg %	Adenosine equivalent g per ml		Pentose mg per 100 ml		
			Whole blood	Plasma	A	B	P
Muscle and bone injuries	Slight	2·63	150	21	4·68	5·00	2·17
	Moderate	3·22	110	19	4·08	5·14	2·23
	Severe	3·80	150	18	5·55	6·07	2·25
Abdominal and thoraco abdominal injuries		4·23	150	14	5·05	6·06	2·67
Burns	Slight	4·80	170	26	4·51	5·40	2·41
	Moderate	3·28	130	19	4·70	5·24	2·32
	Severe	3·58	100	19	5·02	5·56	2·19
Haemorrhage		3·01	150	22	5·42	6·14	2·35
Blood pressure	110 mm Hg and over	2·47	110	21	4·47	4·60	2·19
	101 130 mm Hg	3·20	130	17	4·62	5·24	2·23
	100 mm Hg and below	4·25	170	21	5·56	6·18	2·36
Pulse rate beats/min	Under 70	3·47	—	15	5·00	5·55	2·00
	70 100	2·90	140	18	5·08	5·18	2·18
	100 and over	4·74	170	22	5·70	6·54	2·10
Controls		2·96	138	11	3·69		2·07

TABLE III

Mean values for haemoglobin haematocrit, blood sugar, non Protein nitrogen and amino nitrogen levels for the different groups of cases
The criteria for the classification of the cases are as for Tables I and II

		Hb (Sahli)	Ht	Blood sugar mg per cent	N P N mg per cent	Amino N mg per cent
Muscle and bone injuries	Slight	86	42	137 0	50 1	3 65
	Moderate	70	79	152 0	48 4	3 25
	Severe	60	74	168 0	43 7	3 06
Abdominal and thoraco abdominal injuries		84	40	168 5	40 0	3 16
Burns		60	48	109 0	41 4	—
Haemorrhage	Slight	85	43	148 0	47 6	3 55
	Moderate	73	75	151 0	49 8	2 97
	Severe	73	36	177 0	43 2	3 17
Blood pressure	140 mm Hg and over	83	41	123 0	47 3	3 38
	101 139 mm Hg	80	40	152 4	48 0	3 32
	100 mm Hg and below	77	30	170 0	47 8	3 09
Pulse rate beats per min	Under 70	80	35	131 0	59 7	2 79
	70 100	83	43	143 0	45 8	3 36
	100 and over	74	34	172 6	48 2	3 02
Controls		102 6	46	98 3	—	3 3

TABLE IV

Mean values for the blood levels of the various components of the " nucleotide complex " in the different groups of cases   The criteria for the division of the cases are the same as in Tables I and II   The control mean values are shown for comparison

		Inorganic phosphate mg %	Adenosine equivalent g per ml		Pentose mg per 100 ml		
			Whole blood	Plasma	A	B	P
Muscle and bone injuries	Slight	2 03	150	21	4 68	5 00	2 17
	Moderate	3 22	140	19	4 68	5 14	2 22
	Severe	3 89	150	18	5 55	0 07	2 25
Abdominal and thoraco abdominal injuries		4 23	150	14	5 05	0 06	2 57
Burns		4 86	170	26	4 51	5 49	2 41
Haemorrhage	Slight	3 28	130	19	4 76	5 24	2 32
	Moderate	3 58	100	19	5 02	5 56	2 10
	Severe	3 64	150	22	5 42	0 44	2 35
Blood pressure	140 mm Hg and over	2 47	140	21	4 47	4 00	2 10
	101 139 mm Hg	3 20	130	17	4 62	5 24	2 23
	100 mm Hg and below	4 25	170	21	5 50	0 18	2 36
Pulse rate beats/min	Under 70	3 47	—	15	5 00	5 55	2 00
	70 100	2 89	140	18	5 08	5 18	2 18
	100 and over	1 74	170	22	5 70	0 54	2 40
Controls		2 06	138	11	3 09		2 07

TABLE V

Comparison of the means for the various blood constituents from the values given in Tables III and IV

Blood constituent	Control		Trauma		Difference d	S E of difference (σ_d)	Ratio d/σ_d
	Mean	C O V	Mean	C O V			
Hb per cent	102 5	6 300	78 7	19 705	23 8	1 450	16 31
Ht per cent	47 6	6 543	38 7	21 449	8 9	0 704	11 21
Blood sugar (mg per cent)	98 3	18 870	152 6	42 908	−54 3	6 672	8 14
N P N (mg per cent)			47 5	29 891			
Amino N (mg per cent)	3 3	27 104	3 3	19 105	0 0	0 110	—
Inorg P (mg per cent)	3 0	—	3 4	45 564	−0 4	0 161	2 48
Blood adenosine (µg /ml)	137 9	35 894	146 4	36 800	−8 5	0 715	1 27
Plasma adenosine (µg /ml)	10 0	36 351	19 2	40 846	−8 3	0 906	9 16
Pentose A (mg per cent)	3 7	14 803	5 0	21 000	−1 3	0 114	11 40
Pentose B (mg per cent)			5 6	18 720			
Pentose P (mg per cent)	2 1		2 3	13 749	−0 2	0 032	0 25

refrigerator when the extracts were filtered and the inorganic P of the filtrate determined   The remainder of the filtrate was stored in the refrigerator until transferred to the home laboratory   The adenosine equivalent and the pentose content of these filtrates was determined by the methods used for plasma   The creatine content of the filtrates was determined by the method of Eggleton and others (12)   Parallel determinations of the dry weights of the specimens were also made and the results expressed in mg per g dry wt

3   *Urine*   Only the usual clinical tests were performed

RESULTS

Blood chemistry

The full clinical and chemical findings in each individual case have been deposited with the Medical Research Council   Here we wish to discuss the mean values for the different fractions of the blood which have been estimated   These are shown in Tables III, IV and V , results obtained on acetone precipitates are not included in these tables and will be considered separately   Table III shows the mean values obtained for the Hb, Ht, blood sugar, NPN and amino-N levels in the different groups of cases and Table IV is a similar table for the components of the nucleotide complex Table V gives a statistical comparison between the values obtained in the control subjects and those in the whole group of injured patients   The coefficients of variance show that the values in the latter group are much less homogeneous than in the control group   Before considering the different blood constituents individually it should be pointed out that the means of the trauma group, comprising all the patients, are weighted towards normality by the presence in the group of slightly injured patients who gave values not grossly different from the controls

Hæmoglobin and Hæmatocrit levels   In civilian cases it was found that the post-traumatic state in man is associated with hæmodilution (18) Hæmoconcentration in that series was only seen after burns and abdominal catastrophies   Here, burns and abdominal injuries were always complicated by hæmorrhage, and hæmodilution occurred, but to a less extent than in the other groups of injury where the fall in Hb and Ht was proportional to the severity of the injury (Table III)   Hæmodilution continued for some days after injury despite transfusion   A more complete description of the hæmatological changes in this type of case has already been given (9)

Non-protein nitrogen   After injury the blood NPN (Table V) was slightly higher than usual in normal people of the same age group   This initial increase was not large and was roughly similar in each group (Table III) so that neither the severity of the injury nor the degree of hæmorrhage appeared to be determining factors

Blood sugar   In certain groups the blood sugar level was much raised and a definite gradation was seen according to the severity of the injury, the degree of hæmorrhage, the fall in blood pressure and the rise in pulse rate (Tables III and V)   The full explanation of this is not known   It might be thought that the sweet drinks which many of the patients received before we saw them would be a major factor   This point was investigated in some detail but a definite relationship could not be established   Furthermore, the more severely injured patients who showed the largest increases had usually had the least food and drink   This factor can, therefore, only be a contributory one   *In vitro* studies showed that pentose does not interfere with the estimation of the blood sugar by the method used so that the changes in the plasma pentose described below could not directly account for any of the increase in blood sugar observed   Since the liver is capable of synthesising hexose from d-ribose (36) the possibility that some of the excess blood glucose is derived from ribose compounds released from injured tissues may have to be considered

Amino nitrogen   The plasma amino-N of the injured patients did not differ from the level in the control subjects (Table V), nor were any of the different groups characterized by high mean values (Table III)   This was rather unexpected since increases in this fraction have been reported in animals after several forms of injury (25), (13), (22)   An increase in man after operation has also been reported (29) although more recently (34) this could not be confirmed   Further study of our results showed that there was a relationship between the amino-N level and the blood sugar concentration, the former varying inversely with the latter   The control series did not show this relationship, possibly because of the narrower range of the blood sugar values in these subjects   *In vitro* tests showed that this was not due to the interference of glucose in the method used for determining the amino-N   A similar relationship has been shown in man (23, 24) for administration of glucose lowered the level of the glutamine-like substance which is the main constituent of the plasma amino-N (20, 21)

Nucleotide and phosphate fractions   To discover alterations in the distribution of nucleotide in the body after trauma the various components of the nucleotide complex (Inorg P, " 7 min " P, pentose, adenosine) must be studied individually

Mean values for the control and trauma groups (Table V) show that those for the plasma adenosine and pentose (A and P) fractions in the trauma group are greater than those for the controls and that, statistically, the difference is highly significant   The increase in the inorganic P level in the trauma group is also statistically significant but to a less degree   Table V also shows that of the six estimations which give information of nucleotide metabolism the mean values for the trauma group are increased in five   This alone is significant, for the chances of such a combination occurring fortuitously are approximately 1 in 10   The biological significance of these

TABLE VI

Percentage of cases in each group which had blood levels greater than the control mean + 2σ

		Inorganic phosphate	Adenosine equivalent		Pentose	
			Whole blood	Plasma	A	P
Muscle and bone injuries	Slight	17	5	43	38	10
	Moderate	22	2	46	35	22
	Severe	40	10	56	70	20
Abdominal and thoraco abdominal injuries		44	0	33	66	50
Burns		33	0	75	33	33
Haemorrhage	Slight	23	0	45	40	29
	Moderate	30	10	50	52	17
	Severe	43	7	57	57	28
Blood pressure	140 mm Hg and over	7	0	66	28	33
	101 139 mm Hg	23	2	39	37	21
	100 mm Hg and below	45	12	50	70	27
Pulse rate beats/min	Under 70	—	—	—	—	—
	70 100	14	0	11	44	11
	100 and over	57	10	63	76	31
Controls		4	1	4	4	2
Control mean + 2σ		4.04 mg %	240 µg/ml	19 µg/ml	4.60 mg %	2.67 mg %

+ 2σ = two standard deviations of the control group

results is increased when it is remembered that the trauma group contains cases of all grades of severity, and that in it no attempt has been made to distinguish between major and minor injuries   Nor have we separated out those cases which were transfused before examination   Case 122 (Appendix I) and Table VII show the effect of transfusion on these constituents   The trauma group is, therefore, quite heavily weighted towards normality

Differentiation according to the severity of the injury has been made in the same way as before and the means for the different groups are shown in Table IV   An easier way of studying the distribution in the various groups is to consider the percentage of cases in each group giving values greater than the control mean by more than twice the standard deviation of the controls (Table VI)

Examined in this way the values for the inorganic P and total pentose (A and B) contents of the plasma all show a distinct gradation according to the severity of the injury   The number of cases giving high values for the phosphorylated fraction of the plasma pentose is appreciable, but no definite gradation according to the type of case is seen   The gradation of the pentose values might have been more striking if more untransfused cases of severe injury had been obtained   The mitigating effect of transfusion on the change in the pentose values in cases of severe muscle injury is shown in Table VII   By this method of examination the adenosine equivalent values for the whole blood show little change   On the other hand the percentage of abnormal values for the adenosine equivalent of the plasma, whilst being high in many of the groups, do not show such definite gradation according to the severity of the injury   Trauma appeared to have no significant effect upon the " 7 min " P level of the plasma

TABLE VII

Effect of transfusion in cases of severe muscle injury on the plasma pentose fractions   All the estimations were made on specimens of blood obtained prior to surgical operation   Specimens from the transfused cases were obtained at varying intervals (2 to 12 hr) after the transfusion of stored blood or plasma

| | Controls | Total cases | Mean values (mg per 100 ml) | |
			Not transfused	Transfused
Pentose A	3 7	5 51	5 81	4 72
Pentose B		6 00	6 36	5 15
Pentose P	2 1	2 26	2 40	1 85
No of cases in group	152	30	22	8

TABLE VIII

Mean values for inorganic phosphate, adenosine equivalent and pentose A and P values obtained on the acetone precipitates of plasma from normal subjects and battle casualties   Number of specimens in each group given in brackets in first column

Plasma constituent	Inorg P mg per 100 ml	Adenosine equiv μg per ml	Pentose A mg per 100 ml	Pentose P mg per 100 ml
Control group (27)	2 41 ($\sigma = 0$ 5)	11 ($\sigma = 3$)	2 55 ($\sigma = 0$ 37)	2 19 ($\sigma = 0$ 30)
Trauma group (55)	3 05 ($\sigma = 1$ 07)	15 ($\sigma = 12$)	3 81 ($\sigma = 0$ 99)	2 15 ($\sigma = 0$ 28)
Value of t from comparison of means	2 86	1 74	6 26	0 423

σ = Standard deviation

The results obtained on the acetone precipitates are shown in Table VIII   The number of casualties examined in this way is too small to be subdivided into the groups used above but some conclusion can be drawn from a study of the means   Firstly it is clear the values obtained for the different plasma fractions in the injured patients are much more scattered than in the control subjects   In this respect these figures resemble those obtained on the trichloroacetic acid extracts   Secondly a statistical comparison of the means shows that the trauma group is significantly different from the controls only in respect of the inorganic P and pentose A values   Again it must be emphasized that the trauma group is composed of patients with injuries of all grades of severity   Nevertheless, although there may be some objection to comparing the two groups in this way, the conclusions obtained are of some theoretical interest as will be shown in the discussion

An inherent difficulty in our work was that we had no means of knowing the levels of these compounds in the blood of the casualties before injury From previous work it seemed that although these levels vary widely from individual to individual, in the same individual they remain fairly constant Consequently it would be possible for a person whose normal levels were at the lower limit of normality to show an appreciable increase after trauma and yet not appear abnormal when the results are examined as above In a small series of cases in which specimens were collected before and after abdominal operations there was some evidence that this did occur   To try and overcome this difficulty further specimens were taken in 25 battle casualties in the hope that as they recovered their plasma levels would return to their pre-injury state   The number of cases observed in this way is obviously too small for definite conclusions to be drawn   However, it is interesting to observe (Appendix I) that, on the whole, the levels of these blood constituents do tend to vary with the condition of the patient, reverting

Relationship between the clinical state of the patient and the results of blood and plasma analyses

Case No	Description of wound and progress of patient	Time since injury—hrs	Serosity	Haemorrhage	Hb %	Ht %	Blood sugar mg %	N.P.N mg %	Amino N mg %	Inorg P mg %	Ad equiv γ/mL B	Ad equiv γ/mL P	Pentose A	Pentose B	Pentose P	Vol of fluid transfused in mL	Interval between transfusion and taking specimen (hrs)
1*	G.S W. Comp fracture left tibia and fibula. B.P 70/2 B.P 90/35	5 6	++	+++	98 78						140 180	8 41	6 66 3 73	7 97	2 50 1 97	† 2250 Blood	12 15
3	Perf abdomen laceration liver and contusion of kidney B P 90/80 Laparotomy Slight jaundice Condition satisfactory	6 1°6	++	+	89 86	10 30	91 0 127 0	36 7 57 0	3-42 3-47	3 92 2 01	230 140	15 56	5 50 3 65	5 74	2 13 2 46	† 1100 Blood	91
8	Pen G.S W thigh Sacrum fractured Extra-peritoneal tear of rectum B.P 50/ Left inguinal colostomy Never recovered from op Died	12 27 45	+++	++	73 95	26 42	76 0 85 0		7 22		110 160	15 23	3 62 4 27	4 54	2 00 2 03	‡ 1700 Plasma † 1100 "	6 13
9	G.S W thigh. Femoral art, torn B P 90/50 Operation Improving Impending gangrene of leg	10 22 44 138	+++	++	70 61 61	32 29 25	95 0 180 0 129 5	42 0	3 70 2 63	7 15 4 24 2 93	180 160 200	37 19 22	18 0 5 00 3 70	5 71	1 84 2 15		
11	Comp fracture femur Ruptured urethra B.P 70/50 Improving	4 13	+++	++	80 80	38 33	137 0 170 0	37 4	3 98	0 06 4 24	80 230	9 15	3 11 3 08	6 25	1 77 2 15		
23	Pen. Abdo Fractured sacrum B P 200/110 Jaundiced Slight improvement Less jaundice Improving	31 108 174	++	+	56 60 65	25 25 27	135 0 83 5	63 0 58 5		3 95 2 33	230 220	19 8	4 13 3 40		2 09	‡ 1700 Blood	18
31	G.S W Both buttocks Fractured sacrum Perforated rectum B P 75/60 Improving	9 46	+++	+++	71 80	33 50	177 0 208 0	33 8	3 5,	4 50	230 160	19 10	4 14 6 37	7 14	2 30 1 86	† 7600 "	Continuously between specimens
39*	Comp fracture femur B.P 75/45 Improving	? 2+30	+++	++	54 53	24 21	174 0 130 5	68 0	2 08	3 37 2 11	230 220	6 6	2 94 3 39	6 10	1 86 2 22	‡ 568 Plasma † 568 "	6 31
43	Bilat. comp fracture femur B.P 70/60 Jaundice Improving	5 31	+++	++	61 60	30 28	317 0 157 5	55 0 55 1	2 38 4 61	3 34 2 24	230 220	23 16	0 67 4 63		2 05 2 17	† {1100 " 568 "	{40 43
46	Comp fracture tibia and fibula B.P 105/80 Improving	4 19	++	+	86 65	11 34	250 0 103 0	5 3 17 5			130 180	30	0 68	8 41	3 05	† 1100 Blood	1
56*	Pen G.S W Thigh vein severed B.P 90/60 Bilateral spasm Femoral arteries. Traumatic aneur. Died	9 25 47 97 105	+++	++++	40 75 80 65	20 35 34 28		71 4 83 0 232 0	4 72 4 46	1 85 4 01 4 67		20 18 1 8	2 24 4 28 4 05 7 90	5 16 4 35	1 82 2 07 2 33 2 61	‡ {1700 Plasma 1100 " † 2250 Blood	{8 2 12

Case No	Description of wound and progress of patient	Time since injury—hrs.	Severity	Haemorrhage	Hb %	Ht. %	Blood sugar mg %	V.P.N mg %	Amino N mg %	Inorg P mg %	Ad equiv μg/mL B	Ad equiv μg/mL P	Pentose mg % A	Pentose mg % B	Pentose mg % P	Vol of fluid transfused in ml	Interval between transfusion and taking specimen (hrs.)
61	Pen. abdo. Perf. small bowel. Fairly satisfactory	19 / 50	+	+	101 / 93	63 / 59	224.0 / 101.0	51.6 / 42.3	.14 / 1.79	2.64 / 2.51	140 / 90	.3 / 1.3	6.13 / 6.00	5.09	2.01 / 2.73	†{1100 Plasma / 508 0.9% NaCl}	.0
63	Multiple G.S.W. Comp. fracture femur and tibia. Post. tib. art. and vein severed. Condition very poor. L8Q	16 / 65 / 80	+++	++	66 / 65 / 63	28 / 30 / 23	193.0	46.5 / 65.0 / 89.0	3.31 / 4.68 / 3.72	3.70 / 1.20	90 / 300	19 / 20	3.60 / 4.00 / 3.70	—	3.31	‡ 2950	3
70	Traum. amp. lower ⅓ tib and fib. Condition fair. Died	6 / 28 / 39	+++	++	83 / 76	43 / 30	260.0	18.6 / 87.0	2.21	5.66 / 3.20	130	19 / 19	6.23 / 4.51	8.06	2.63 / 1.90	‡ 2950	3
76	Traum. amp. foot	11 / 13	++	++	73 / 73	35 / 35	116.5 / 109.0	31.7 / 30.0	2.31 / 3.69	4.38	170 / 180	22 / 18	4.47 / 3.70	4.37	1.58 / 1.47	‡{508 Plasma / 1700 Blood}	1
83	Comp. fracture femur. D.P. 70/40. Satisfactory	6 / 20	+++	++	78 / 75	49 / 33	90.0 / 68.0	37.0 / 18.0	3.65 / 4.62	1.90 / 2.00	230 / 210	16 / 15	5.10 / 3.65	4.00	2.51 / 1.07	†{1100 Plasma / 1100 Blood}	⅓ 14 / 14
86	Comp. fracture tib and fib. D.P. 140/80. Unconscious. Fat embolism. Died	12 / 38 / 63	++	++	50 / 59	22 / 23	87.0	81.6 / 82.0	1.43	1.72	160 / 190	10	6.91	—	1.08	‡ 2950 Plasma / 1100	6 / 14
93	Traum. amp. below knee. Satisfactory	3 / 21	+++	+++	90 / 48	96 / 20	467.0 / 101.6	40.6 / 56.3	2.14 / 2.80	1.29 / 1.92	170 / 250	19 / 15	6.88 / 4.90	8.13	2.35 / 1.72	†{1400 Plasma / 1100 Blood / 2250 Plasma}	3 / 3
91	Traum. amp. both feet. Satisfactory	3 / 18	+++	+++	60 / 70	36 / 31		85.1	3.72		160 / 130	23 / 19	6.88 / 4.06	8.00	2.99 / 1.39	†{1100 Plasma / 250 Blood}	9
108	G.S.W. buttocks and legs. Satisfactory	4 / 19	+++	++	55 / 57	35 / 33	144.5	46.2 / 5.1	2.51 / 3.18	2.62 / 2.2	110 / 110	12 / 13	4.00 / 4.54	4.93	2.20 / 1.74	‡{1100 Plasma / 1100 Blood / 1100 Plasma}	3 / 9
110	Pen. chest. Haemothorax. Satisfactory	3 / 41	++	+	75 / 73	40 / 35	118.0 / 1.15	94.4 / 43.0	2.30 / 3.12	1.47	100 / 110	12 / .3	6.62 / 3.07	1.74	1.73 / 2.07		
118	Comp. fracture humerus. D.P. 140/95. Satisfactory	10 / 11	++	++	88 / 85	45 / 40	107.0 / 110.5	110.0 / 53.1	2.71 / .98	2.63 / 2.63	110	8	5.90	—	3.83		
122	G.S.W. Thigh. B.P 85/50. Over transfusion 6 pints in 1 hr. B.P 70/15	10	+++	++	69 / 13	33 / 10	222.0 / 107.0	35.3 / 20.8	3.00 / -.54	3.29 / 1.76	140 / 90	8 / 8	8.25 / 6.81	8.69	2.76 / 1.87	†{...}	
20*	Laceration of liver. B.P 138/80. Condition fair. Condition good	3 / 23 / 63		+		45 / 43 / 41				3.88 / 3.37 / 3.10			3.70 / 3.61 / 4.47				
28*	Krentzmann. Buttock wound. Condition satisfactory	4 / 28	+++	++		45 / 17	180.0 / .17			1.80 / .17		24 / 1.	5.50 / 5.17			† 508 Plasma	22

G.S.W = Gun shot wound   Pen = Penetrating   Amp = Amputation

* = Analyses performed on acetone precipitated plasma   † = Transfused between initial and subsequent specimens

‡ = Transfused before initial specimen

TABLE IX

Results of the analysis of muscle specimens removed at the time of operation   "Abnormal" muscle = grossly damaged, non viable muscle , "normal" muscle = viable muscle removed from the wound close to the site of abnormal muscle   A and B show the total pentose content of specimens of completely normal human muscle   Results are expressed in mg per g dry wt

Case No	State of muscle	Creatine mg /g	Inorganic phosphate mg /g	Adenosine equivalent mg /g	Total pentose mg /g
1	Abnormal "	16 0	3 50	5 10	6 90
2	Normal "	6 66	2 96	3 24	3 38
4	' Normal '	12 0	7 36	6 79	6 57
4	Abnormal '	12 6	6 55	2 41	3 73
10	Abnormal '	9 64	2 91	1 26	2 00
15	Abnormal '	8 25	2 87	2 15	2 55
18	Normal	7 15	2 45	3 20	2 75
18	Abnormal	5 24	2 19	1 67	1 95
26	' Normal "	2 75	2 20	2 30	2 20
35	Normal "	14 8	3 29	2 92	4 63
35	Abnormal "	13 8	3 43	4 76	4 62
43	' Abnormal '	2 42	2 75	0 97	1 12
46	' Abnormal "	2 00	2 50	1 69	1 13
65	Normal "	7 37	2 79	8 01	3 14
66	Abnormal '	7 59	3 42	3 26	1 48
70	' Normal '	15 2	4 15	8 92	6 49
70	Abnormal '	19 3	7 66	6 40	6 47
81	' Abnormal	5 24	3 24	5 72	3 53
86	' Normal "	13 6	7 22	6 52	6 83
86	' Abnormal '	13 05	9 58	5 05	5 74
114	' Abnormal "	4 88	6 72	2 82	
122	" Abnormal "	16 3	3 30	9 23	3 08
Mean	" Normal "	9 9	4 1	5 3	4 5
Mean	" Abnormal "	9 8	4 3	3 7	3 4
—	A	—	—	—	11 85
—	B	—	—	—	11 40

towards normal in those who recovered and showing increasing abnormality in those who died   Transfusion was again an interfering factor as is revealed in Appendix I

Muscle chemistry

Analyses were carried out on 22 specimens of muscle removed at operation   Fourteen of these specimens were definitely abnormal   The remainder were classed as 'normal," the criteria being bleeding and contraction on cutting   They were, however, removed from near the site of injury and one cannot vouch for their complete normality   The results (Table IX) showed that whilst the differences between the two groups were not statistically significant the values obtained for the adenosine equivalent and total pentose concentrations in the damaged muscle did show a tendency to be lower than in the so-called " normal " muscle   This difference was best seen when it was possible to compare two specimens of muscle from the same case   Comparison of the values obtained for the total pentose concentration in these cases with those obtained on two completely normal specimens of human muscle (A and B, Table IX) strongly suggests that all these specimens were abnormal and gave values much lower than are found in normal muscle   Later observations on normal muscle indicate that the interpretation of these results is almost certainly that the nucleotide levels of muscle in and around the obviously damaged area are much reduced

DISCUSSION

Whilst mainly interested in the effect of trauma on the blood chemistry certain clinical features of these patients attracted our attention   For example the resistance of the trained soldier to trauma was obviously greater than that of the average civilian   One repeatedly saw much less general bodily disturbance after injuries of a given degree than we were accustomed to in civilian practice   It has been pointed out that the pain associated with these severe injuries is often not severe (2)   The main complaint was thirst, often very intense and lasting 36-72 hours after the injury   This thirst was not relieved by transfusion and often no attempt was made to relieve it by giving water by mouth, chiefly on the grounds that it led to vomiting and so interfered with anæsthesia   We were not convinced that this was a valid objection and the slow and continuous oral administration of water should be tried

Plasma and blood appeared equally effective in raising the blood pressure Rigors were very frequent with both   At times too much stress was laid, in our opinion, on this form of therapy and time wasted which might have been better spent in operative treatment   The value of early, radical operation which had become so apparent from our earlier work, was not always appreciated, chiefly due to failure to realise the extent of the tissue damage   The clinical estimation of the amount of damaged tissue by Grant's method is extremely difficult, for the appearance of the wound often

gives little indication of the extent of the damage   The post-mortem findings in our series (Appendix II) showed very clearly the large amount of muscle damage which is associated with injuries due to high velocity missiles The amount of tissue damage and the degree of hæmorrhage appear to be the factors which determine the state of the patient   The estimation of the amount of blood loss is as difficult as that of the amount of injured tissue but our observations suggested that the latter is the more important of the two

Although fat embolism is a common pathological finding in patients dying after trauma the clinical manifestations of the condition appeared more frequently in military than in civil cases   While trauma seems to be the cause of this condition its amount need not be very great , in one fatal case there were only 3 small wounds which did not penetrate the deep fascia The condition is not necessarily fatal and one of our cases recovered after being in coma for a week   Anæsthesia seemed to play a part in precipitating the condition in some cases

Estimations of inorganic P, " 7 min " P, pentose and adenosine levels of the blood all give information of the nucleotide distribution although in the case of the inorganic P the information is indirect   Simple comparison between the controls and the trauma group shows that blood from the latter group contains more of these nucleotide fragments and that this difference is statistically significant, despite the trauma group being diluted by minor injuries and by cases that had been transfused   This enhances the biological significance of these results as does the distinct relationship between the clinical state of the patient, the severity of his injuries and the changes in the nucleotide components of the blood   Trauma in man would, therefore, seem to be associated with an increase in the various nucleotide components in the blood stream   Our results show the state of affairs mainly between 2 and 12 hours after injury   It is not known whether or not the maximum change in these blood components occurs during this period   In view of all these uncontrollable variables it is perhaps remarkable that such definite results have been obtained   For the inorganic P fraction there are many possible sources and therefore estimations of this fraction can only give indirect evidence of alterations in nucleotide metabolism   The volume of injured tissue seems to play a greater part in producing this excess than the degree of hæmorrhage although it is known from animal experiment that simple hæmorrhage will raise the inorganic P level of the plasma (31) Another factor which must certainly play a part is the depression of renal function   However, the size of the increases so soon after the injury suggests that, of itself, this is not a main factor   Possible tissue sources for this phosphate are numerous but in muscle, ATP forms the main single phosphorus-containing component and much of that contained in other compounds is ultimately derived from it   From our own results and those of others (6, 7, 28, 30) it is known that ischæmia of muscle leads to the

breakdown of ATP with the liberation of inorganic P   It is probable that the increase of the plasma inorganic P is, at least in part, a reflection of this catabolic process

The increased amounts of circulating adenosine and pentose both probably arise from the damaged tissue   Animal experiments (37) have shown that depression of renal function does not increase the adenosine equivalent of the blood   Experiments on man have shown that morphine is not responsible for these increases   These compounds may also be released into the plasma from the erythrocyte where the concentration of adenyl compounds is relatively high, a possibility made more likely by the increase in erythrocyte fragility after trauma (35)   However, hæmolysis was only infrequently observed and such specimens were discarded   Furthermore, although the increases in the adenosine equivalent were almost confined to the plasma, slight increases in the adenosine equivalent of the whole blood were found and the increase in the mean value for this fraction in the trauma group as compared with the control suggests that hæmolysis could only be a minor factor   The possible effect of the hyperglycæmia seen in these patients on the estimation of the pentose levels (32) has been fully dealt with elsewhere (17)   Dr M Bielschowsky found that the changes in the pentose levels could not be accounted for by variations in the glucose content of the blood

By a process of exclusion, therefore, it would seem that the majority of these excess nucleotide components are derived from the damaged tissue   This can only be proved by examination of this tissue   Our results certainly show that the nucleotide content of muscle in the injured area is less than in normal muscle but again many factors play a part in this decrease   The mere fact that the content is low does not mean that any has been released into the circulation   The results here are, however, comparable with those obtained in animal experiments (4) which demonstrated the release of nucleotide fragments from ischæmic muscle, but these experiments had to be carefully controlled by methods which could not be applied to these human specimens

The question remains as to whether the different substances we have estimated are present in the plasma, free or in a combined form   If, as seems probable, the increases in these substances are derived from an alteration in nucleotide metabolism in the damaged tissue then they will exist either as ATP itself or as a compound, or mixture of compounds, intermediate between this mother substance and its basic fragments   This question is important because although the excess is in the plasma and therefore potentially active, there is a great difference between the toxicity of the basal compounds such as adenosine and inosine and the fully phosphorylated compounds such as ATP and ITP   It is not possible from our data to make any definite statement on this subject   The results suggest that if these compounds are present in a combined form they are not present as fully

phosphorylated nucleotides   Evidence for this is seen in the large increase in inorganic P and lack of change in the " 7 min " P fractions   The inability of the phosphorylated (nucleotide) pentose fraction to account for the major part of the total pentose is also in favour of this   It has been pointed out here that it is only in the case of the inorganic P and total pentose that the results obtained on the acetone precipitated plasma of the casualties differ from the controls   Whilst the exact nature of the compounds precipitated from plasma by acetone is not clear, it is known that the more fully phosphorylated compounds are more completely precipitated by this agent Consequently these results are also in favour of the occurrence of the lower compounds although some phosphorylated forms must be present   The increase in the adenosine equivalent of the plasma suggests that the purine ribose linkage is preserved since adenine is not estimated by this test   When the values for the adenosine equivalent and pentose of the plasma are compared it also seems likely that deaminated forms of these compounds are present The general conclusion is that the plasma levels are increased by an influx of nucleotide breakdown products in which a mixture of nucleosides predominates

This general conclusion is in close agreement with previous results for we have shown that the adenosine equivalent of the blood is increased after limb ischæmia, direct muscle trauma, burns and other forms of injury in the rabbit (37, 38, 40) and after experimental limb ischæmia in man (39)   We have also shown that these types of trauma are associated with increases in both the total pentose and its phosphorylated fraction in the plasma of the rabbit and rat (19)   Similar results concerning the plasma pentose (32) and the adenosine equivalent have been reported by others (5, 8, 26) Trauma would appear to be accompanied by an influx of nucleotide breakdown products into the circulation   These substances may play a part in the production of the general bodily reaction to trauma but no definite answer can yet be given to this question

SUMMARY

1   Observations on 177 battle casualties and 164 healthy soldiers are reported   The nucleotide distribution in the blood and in muscle at the site of injury was particularly studied

2   Hæmodilution was a constant finding and except in burns- and abdominal injuries the fall in hæmoglobin and hæmatocrit values was proportional to the severity of the injury

3   Hyperglycæmia was a prominent feature and its degree also varied with the severity of the injury

4   The plasma amino-N level was not raised but bore an inverse relationship to the blood sugar level

5   In the trauma group, considered as a whole, the adenosine equivalent and the total and phosphorylated pentose levels of the plasma trichloroacetic acid extracts were greater than the control levels by amounts which were statistically highly significant   The inorganic phosphate level was also significantly raised after injury   The acid labile phosphate level was unaffected   There was a slight but statistically insignificant rise in the adenosine equivalent of the whole blood

6   The initial values for inorganic phosphate and total pentose, but not for the adenosine equivalent or phosphorylated pentose, showed a gradation according to the severity of the injury

7   Similar determinations on the acetone precipitates of plasma in 55 battle casualties showed that the trauma group differed significantly from the controls only in their inorganic phosphate and total pentose levels

8   Follow-up observations on 25 patients showed that the levels of the blood nucleotide fractions vary with the clinical condition of the patient

9   Twenty-two specimens of muscle from the site of injury were examined   No significant differences between injured and nearby " normal " muscle were found but the results as a whole almost certainly indicate that the nucleotide content of the muscle in and around the wounded area was much lower than in normal muscle

10   Considered as a whole these findings support the view, already established in animal experiments, that trauma in man is associated with a redistribution of nucleotides within the body

11   Certain clinical features of severely injured men are discussed and a summary of the post-mortem findings is given in Appendix II   It is noted that the volume of muscle damaged may be commonly underestimated   The extent of this damage is probably of great importance in determining the clinical condition of the patient

APPENDIX II

Post mortem investigations on battle casualties

Nineteen cases in our series of 177 battle casualties died and a complete autopsy was performed on 16 of these   The causes of death in these cases were as follows —

Cause of death	No of cases
Muscle trauma and hæmorrhage	6
Hæmorrhage into pleural cavity	2
Fat embolism	3
Traumatic anuria	1
Tension pneumothorax	1
Peritonitis and muscle trauma	1
Aspirated vomit	1
Not discovered	1

This table emphasizes the importance of muscle trauma and haemorrhage in producing death One of our main interests in carrying out post mortem investigations on these cases was to determine the amount of muscle damaged by the injury In every case we found that the amount of muscle damaged by these skeletal injuries was very much greater than we had anticipated This was especially true of buttock wounds where the amount of damaged muscle was often enormous The volume of internal haemorrhage was also greater than had been imagined before death

The commonest cause of death was muscle trauma combined with haemorrhage In these cases the volume of damaged muscle was never less than 2 fistfuls whereas the degree of haemorrhage varied from slight to severe which may indicate that muscle trauma is the more important factor It also seemed that the only way of assessing the amount of damaged muscle in a patient is to draw on the experience gained from autopsies in similar cases

In the 3 fatal cases of fat embolism, 2 had injuries below the knee necessitating amputation and the other which was of the pulmonary type had 3 superficial wounds not extending below the deep fascia Fat was demonstrated histologically in all the organs and especially in the lungs, which were very oedematous Small amounts of fat were found in the lungs, but not the other organs, of all but 3 of the remaining fatal cases

Every case showed some pulmonary abnormality, usually oedema (14 cases) Both the degree of muscle injury and the volume of fluid transfused seemed to influence the severity of the oedema, but it was not clear from the available data which was the more important Other common changes in the lungs were alveolar haemorrhage (6 cases) and patchy collapse (8 cases)

In addition to the one case of traumatic anuria the kidneys were histologically abnormal in 5 other cases They showed varying degrees of dilatation of the convoluted tubules with flattening of the epithelium and amorphous material in the lumen No pigmented casts were seen These 5 cases all had considerable amounts of damaged muscle ranging from 2 to $3\frac{1}{2}$ fistfuls, except for one in which the liver was extensively damaged

The main impression gained from these post mortem studies was the large amount of muscle damage which accompanies injuries of this type and its importance in determining the outcome of the case

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DEPOSITION OF ADIPOSE TISSUE BETWEEN OCULAR MUSCLE FIBRES IN THYROTOXICOSIS *

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In the course of an investigation of the orbital changes in exophthalmos, the eye muscles of thyrotoxic patients were found to be abnormally rich in material extractable with ether   On chemical analysis of tissue obtained at autopsy   the ether extract of the eye muscles was increased from 8 1% in controls to 13 8% in seventeen thyrotoxic subjects   Histological material from certain of these cases has now been studied to determine the character and location of the abnormality   The human eye muscles are normally rich in adipose tissue cells lying in strands between the muscle fibres In thyrotoxicosis this tissue is increased, as was observed by Silcock in 1886 (3) and studied in detail by Askanazy in 1898 (1) who described it as a "general lipomatosis" of muscles   These observations have since been confirmed and discussed by Schutz (4) and v Zalka (5)   In the present paper the change is examined quantitatively and related to the chemical findings in the same muscles

Method

The orbital contents were removed postmortem   The eye muscles were then dissected clear of fascia and adherent adipose tissue, and split longitudinally into two parts   One part was examined chemically by a method described earlier (2), being first dried and then extracted with ether in a Soxhlet apparatus   The second part was pinned out under slight longitudinal tension fixed in 7% formol saline and frozen sections cut and stained with Sudan III   One estimate of the fat content of the muscle was obtained by expressing the weight of ether extract as a percentage of the wet weight of the muscle   A corresponding histological estimate was derived from measurements of the tissue staining with Sudan III   One, two or three sections from different areas in the muscle were examined The image of each section was projected on to paper on which was traced the outline of the strip of muscle and that of each group of fat droplets   The

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total areas were determined with a planimeter, and that occupied by groups of stained fat droplets was expressed as a percentage of the area of the section

In certain cases a higher magnification of about 20 diameters was also used, so that the outline of the section and that of fat deposits and many of the individual fat droplets could be traced on to graph paper. It was shown by this more accurate but laborious method that the simpler technique of outlining groups of droplets only, over-estimated the area occupied by stained fat by an average of 20%. Most of the stained fat lies in dense clusters of globules (Fig 3), which largely fill the areas occupied by adipose tissue strands in the muscle, and the difference between the methods presumably depends on the closeness of packing of the globules, which appears to be similar in different muscles. Results by the simpler method have therefore been corrected in this proportion

Errors involved in this approximation are likely to be small compared with those due to inadequate sampling of the muscle. Different sections from the same muscle often differed greatly in the percentage of the area stained, and individual values had an average standard deviation of 31% of the mean value for all sections from the muscle. Results in individual cases are therefore variable, and findings in groups of subjects have been examined

By neither method of examining sections were any droplets within muscle fibres recorded, and measurements estimate only an adipose tissue fat content of the muscles. It is therefore possible to distinguish between an increase of fat due to a raised adipose tissue content, and one due to infiltration of the fibres themselves by such material

Results

In the eye muscles from the normal subjects that have been examined, stained adipose tissue globules occupied 5 1% of the muscle area while 8 1% of the muscle was extractable with ether. Taking the specific gravity of fat at 0 8, about 78% of the "fat" determined chemically was recognisable histologically. In the eye muscles from thyrotoxic subjects, both figures were raised, and in about the same proportion. Thus 9 7% of the muscle area was stained, while 15 5% could be extracted with ether, 75% of the chemical "fat" being identifiable in adipose tissue

The results in Fig 1 give average values separately for the different eye muscles, since it is known that these muscles normally differ in their fat content. In individual sections, the chemical and histological estimates were often very discrepant, and even the average values shown in Fig 1 for groups of sections from the same muscle, are liable to substantial uncertainty It will be seen, however, that in general the average values for all muscles are increased in thyrotoxicosis, both in the chemical and histological

estimates of fat, and that these estimates maintain broadly the same
relationship as in normal subjects   A 90% increase in the chemical value
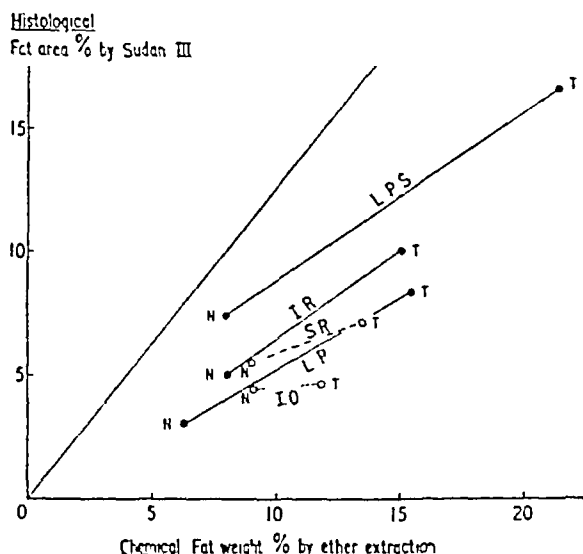in these thyrotoxic muscles was associated with an 85% increase in adipose

Fig 1   Fat content of different orbital muscles as estimated by histological and chemical
methods   For each muscle the line joins two points   T, indicating the mean histological
and chemical fat content of the thyrotoxic muscles, and N at the mean value for normal
muscles   The line through the origin represents equivalence of the estimates on a basis of
specific gravity 0 8 for fat

      L P S — levator palpebræ superioris
      I R   — inferior rectus
      S R   — superior rectus
      I R   — lateral rectus
      I O   — inferior oblique

tissue fat   The latter continued to account for about three-quarters of the
amount of material extractable with ether   It can therefore be stated that
it least the majority of the chemical change is due to an increase in the
amount of adipose tissue fat contained between the fibres of the muscle   It
is not possible to state whether other sites of fat deposition in the muscle
also participate, since the measurements do not account for the whole of the
chemical fraction   The residue may be due to small globules of adipose
tissue fat   to fat or other extractable materials contained within the muscle
fibre   or to volume changes on fixation   Since however demonstrable
globules of adipose tissue fat continue to form a normal proportion of the
total fat in thyrotoxicosis, and are responsible for the bulk of the change,
it seems at least probable that the observed effect may be due solely to an
increase in muscle adipose tissue

It is significant that the change in this respect appears to be a proportionate increase in the normal adipose tissue content   This may be compared with the chemical finding (2) that the increase in the different eye muscles was in each case proportional to the normal fat content of these eye muscles Throughout, the changes are suggestive of an exaggeration of the normal adipose tissue within the muscle rather than of the addition throughout the orbital muscles of an abnormal tissue or material in amounts which would presumably not be directly related to the normal fat content   It is most suggestive that in a study of the orbital tissues, the fat content of the lacrimal gland and that of the orbital adipose tissue mass generally were found to be increased (2)   The findings are consistent with an increased deposition of adipose tissue throughout the orbit

TABLE I

Mean diameter of fat globules in orbital muscle adipose tissue (value in micra)

Muscle	Control subjects	Thyrotoxic subjects
Levator palpebræ superioris	31	24
Superior rectus	42	23
Lateral rectus	31	23
Inferior rectus	41	27
Inferior oblique	42	23
Mean value	37	24

An increase in muscle adipose tissue fat might be due to an enlargement of the globules in existing cells, or to the formation of new cells   In the former case, the mean diameter of globules should be increased, whereas in the latter it might have any value, depending on the mode of development of new cells, but should presumably be decreased while new cells are developing We have, therefore, compared the size distribution of fat globules in thyrotoxic and control muscles   Counts were made on all sections using a micrometer eyepiece and estimating the diameter of 50 to 100 successive droplets in each section   Sections from thyrotoxic and control subjects were numbered and presented in a random order, the observer being ignorant of the nature of each section   It was found (Table I) that the mean diameter of globules from thyrotoxic muscles was 64% that in controls   The distribution of diameters showed an excess of small globules with diameters of from 10 to 60% of the normal average value (Fig 2)   From the areas stained with Sudan III it has been shown that the total volume of fat globules is increased by an average of 85% in thyrotoxic muscles   Since this increased volume is formed by globules of smaller mean diameter, the number of such globules per unit volume of muscle must be increased   The extent of this

increase has been calculated from the diameter distribution and the volume increase   It is found that the number of globules in thyrotoxic muscles is 2 6 times that in controls   The increase in number of adipose tissue cells cannot be stated but, since single cells are unlikely to contain multiple
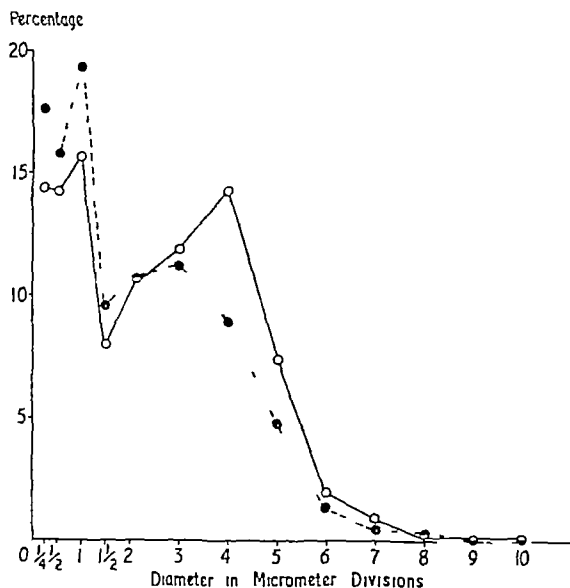
Fig 2   Distribution of fat globule size in orbital muscles showing a relative excess of globules of small diameter in thyrotoxicosis   Open circles and continuous line   normal subjects   Discs and interrupted line   thyrotoxic subjects   (1 micrometer division = 13 5μ)

globules of the size observed   it is probable that the number of cells per unit volume of muscle is correspondingly increased   Moreover, the orbital muscles are increased in weight in thyrotoxic subjects with simple exophthalmos, even when the fat-free muscle weight is considered (2) It is unlikely therefore that the increased adipose tissue is simply a residue or a replacement of degenerated muscle fibres as has been suggested (4, 5)   It represents new tissue increasing the total bulk of the muscles

SUMMARY

1   Strands of adipose tissue cells normally lie between the fibres of the skeletal eye muscles   The quantity of such adipose tissue is increased in thyrotoxicosis by about 85%

2   The increase is proportional to the increase in the ether extract of these muscles reported previously

3   It is associated with a decreased mean diameter of the fat globules, and therefore with an increased total number of adipose tissue cells

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Fig 3   Section of levator palpebræ superioris muscle from a case of thyrotoxicosis, stained with Sudan III and showing copious strands of adipose tissue between muscle fibres (magnification × 16)

LOCAL DEPOSITION OF ADIPOSE TISSUE, EXPERIMENTALLY INDUCED *

By E E POCHIN

(From the Department of Clinical Research, University College Hospital Medical School)

WHEN a body structure decreases in size or is removed, the surrounding tissue will commonly be displaced so as to occupy the space thus emptied The present work was done to answer the question what material or structure is deposited in a cavity created within the body, if adjacent tissues are prevented from filling it ? This problem arose from a study of thyrotoxic exophthalmos (3) when the increased space behind the proptosed eye was found to be largely filled with adipose tissue The total quantity of orbital fat was increased, excessive adipose tissue was found in the skeletal eye muscles (2), and the ether extractable fraction of the lacrimal gland was probably also increased The question then arose whether the increase of adipose tissue caused or resulted from the forward displacement of the eye Was the eye pressed forward by accumulating fat behind it , or did it prolapse forward, for example because the rectus muscles were weak, with adipose tissue secondarily filling the space so created behind it ? The first possibility involved a deposition of fat in several different structures lying in the same anatomical region, and occurring in the course of a wasting disease It was difficult to see what common pathological factor would be likely to cause primary changes of the type observed The second possibility, that the deposition was a more physiological process to fill the increased space behind the eye, could not be considered until it was known what material would fill any such space as it developed

An attempt has therefore been made by animal experiment to create a cavity within the body with as little local trauma as possible, and to study the changes occurring within it It seemed likely that any local excision of tissue would be followed by too great and variable a surrounding tissue reaction and hæmorrhage to give clearcut results It was also necessary that the cavity should not become closed simply by the collapse of surrounding tissues into it In the rabbit however, it was possible to create a suitable cavity, by introducing a small perspex frame subcutaneously

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* Work undertaken on behalf of the Medical Research Council

under the loose and mobile dorsal skin   This frame could be introduced between the skin and the dorsal muscle fascia, with very little trauma to the tissues immediately surrounding it   The contents of the cavity so formed could be studied either by aspirating samples or by killing the animal and dissecting any newly formed tissue lying within the limits of the frame
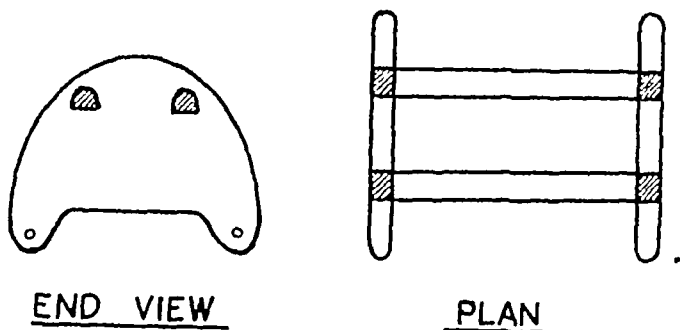
PERSPEX FRAME

END VIEW   PLAN

Fig 1   End and plan view of perspex frame (natural size)

Method

A perspex frame was made by rigidly joining two D-shaped endplates by means of two perspex rods of $\frac{1}{8}$-inch diameter (Fig 1)   The joints between the rods and plates were smoothed off and cemented, and all edges were bevelled off   The frame was inserted subcutaneously by aseptic operation A two-inch incision was made in the mid-lateral line through the skin and down to the fascia covering the muscles of the body wall   The dorsal skin was readily lifted by blunt dissection from the underlying muscle fascia The frame was then introduced into the space so formed so that its base rested on the dorsal fascia in the mid line, the base being shaped to the contour of these muscles   It was held in position by ligatures attached to holes in its base and drawn through the dorsal muscles   The cavity within the frame was thus surrounded by almost normal tissues   Bleeding at the site of the frame was negligible   The skin incision, which was closed by a continuous thread suture, was remote from the position of the frame when the skin fell back into position   The incision in all cases healed by first intention   The air introduced with the frame at operation was ordinarily left, but in certain experiments it was removed by aspiration after suturing the skin   In these cases, the dorsal skin was at first drawn in towards the cavity, forming folds round the perspex bars, but these bars prevented surrounding tissue from filling the cavity   Clinical evidence of infection was seen in one case only and animals showed no sign of pain or discomfort when the area of the frame was palpated   The dorsal skin slipped easily

over the frame and appeared undamaged by it   The animals were given a diet of approximately a half pound of pellets having the following composition —grass meal 30%, bran 15%, ground nut cake 15%, linseed cake 10% barley meal 20%, meat and bone meal 8%, calcium carbonate 1%, sodium chloride 1%   When killed, they were in good condition with the normal subcutaneous and intra-abdominal fat deposits well filled

Samples of the contents of the cavity were obtained by aspiration of fluid through the skin in a few cases, or by dissection at autopsy   The composition of gas samples was not studied   In fluid samples total protein, albumin and non-protein nitrogen were determined by Kjeldahl estimation Tissue samples were grouped according to their origin from the continuous sheet of tissue or capsule forming over the convexity of the frame , from sheets of tissue forming in apposition with the endplates on their outer aspects , or from within the cavity, that is, between the endplates above the level of the dorsal muscle fascia and below the sheet of tissue forming over the convexity of the frame   In most cases, the whole of the tissue from within the frame was received into dried filter paper thimbles in weighed bottles   In some cases an aliquot was sectioned and stained   Tissue samples examined chemically were weighed after drying to constant weight at 105°C and after ether extraction to constant weight in a Soxhlet apparatus Frozen sections were stained with Sudan III

Results

The material formed within the frame has been examined in animals killed at various intervals with the results shown in Table I   The cavity first becomes encapsulated and fills with fluid   This fluid is becoming replaced by solid tissue about eight weeks after inserting the frame   This tissue is initially fat-free but its fat content increases progressively after about the seventh week

To avoid repeated killing of animals, the earliest changes have been studied in more detail by aspirating small samples of the fluid contents of the cavity in a few animals   While this procedure may in itself alter the course of changes, either by reducing pressure or by increasing chances of infection, the animals so studied have shown changes similar on autopsy to those in animals from which samples have not been taken   Unless withdrawn at the time of operation, air introduced with the frame remains for the first week, after which gas is no longer obtained by aspiration

Formation of fluid   Fluid may be obtained from the cavity in small amounts in some cases on the first day after operation, and freely on the second or third day   Such fluid can usually be found, either by aspiration or at autopsy, until about 10 weeks from insertion of the frame, and a small amount was present in one case at autopsy after 131 days   The fluid is commonly of a clear yellow colour, although sometimes containing blood Its protein content is high, and has been found to bear a clear relationship

TABLE I

Contents of cavity within frames at various periods after insertion

Rabbit Number	Period after insertion	Fluid Amount	Protein	Albumin	Tissue Amount	Ether extract		Water as % of fat free
	days	ml	%	%	g	%	g	
8	14	full (over 3)	5 2	3 7	little	1	0 0	89
2	28	full (over 3)	4 3	3 6	little	1	0 0	91
10	36	0 1	4 0	—	2 6	0	0 0	88
11	45	0 0	—	—	1 1	1	0 0	88
9	54	2 7	3 4	2 9	1 5	1 5	0 2	84
6	67	1 8	2 9	2 4	3 7	3	0 1	86
16	74	0 0	—	—	4 6	30	1 4	83
3	80	0 0	—	—	3 5	47	1 6	84
4	131	0 3	2 2	?	3 3	32	1 0	78
1	133	0 0	—	—	full (over 1)	about 90	?	74

Data for rabbits 8 2 and 1 are based on samples and not on total amounts and are therefore incomplete

to the interval since inserting the frame   Thus the mean value in the four animals repeatedly aspirated rose from 3 9% on the second day to a maximum of 4 8% at about the tenth day, and then fell progressively, reaching 4 3% on the twentieth day   The two animals that were again
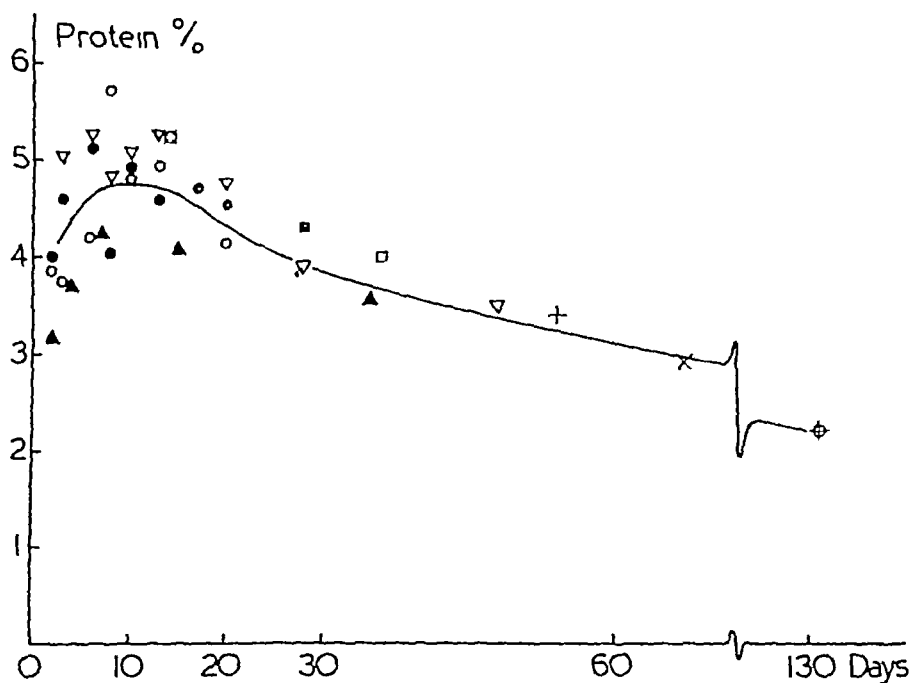

Fig 2   Protein concentration of fluid formed plotted against days since insertion of the frame
    Different symbols represent different animals   Mean plasma protein concentration 6 15%

aspirated and animals killed at later periods, show that this fall continues as in Fig 2, and the fluid sample obtained at 131 days contained 2 2% protein   The ratio of albumin to globulin has not varied consistently with the change in total protein, and has had an average value of 3 3   The non-protein nitrogen has averaged 32 mg per 100 ml   In these animals the plasma protein concentration has averaged 6 2%, with a ration of albumin to globulin of 3 0 and non-protein nitrogen 30 mg per 100 ml   The mechanisms underlying the protein accumulation and removal have not been examined   Although the frame was inserted with sterile precautions and clinical infection of the area was only once observed, mild infection cannot be excluded as a cause of capillary permeability and hence of the high protein content of the fluid   Aspirated samples were plated onto blood slopes and into serum broth in two animals and were found to be lightly infected with

non-pathogenic organisms in the first few days, subsequent samples being sterile   It is thus possible that the rise of protein concentration in the first few days is inflammatory in origin

Formation of tissue   In animals killed within a few weeks of operation, the only new tissue found was a capsule surrounding the frame itself and forming a sac containing the fluid   In those killed later, much or all of the space within the frame was occupied by solid tissue (Figs 3 and 4)   The transition from fluid to solid contents evidently continued from about the fifth to the tenth week and varied in its development in different animals   As judged from animals killed between these times, tissue is formed first round the longitudinal perspex bars, and in the floor of the space and particularly in the centre of the floor   The fluid sac thereby becomes broken into an hourglass shaped cavity   The two roughly conical compartments formed have their bases on the inner aspects of the endplates   As tissue continues to form, the two cavities containing fluid dwindle to shallow spaces lying against the inner aspects of the two endplates

The margins of the new tissue appear to be determined by the way in which the skin sweeps up over the frame from the surrounding areas of back   At the ends, the skin rises onto the frame in a smooth curve from the adjacent area of back, and does not exactly fill the angle between the endplate and the area of back immediately outside the frame   When the skin is removed, it is usually found that the corresponding area against the outer aspect of the endplates is covered with a sheet of tissue which is thickest at its base   New tissue also fills the areas lateral to the frame over which the skin sweeps in a smooth curve onto it   Here the tissue has a greater lateral extent near the endplates of the frame than opposite its centre   In each case therefore the new tissue is closely moulded to the arrangement of the overlying skin, even though the skin is freely mobile over the frame and nowhere fixed to the tissue   The new tissue thus appears to fill the local cavities formed outside and around the frame, as well as the main cavity within it

It is clear on several grounds that the new tissue is not simply laid down around the perspex as a foreign body   Firstly, while the outer surfaces of the frame are surrounded by a capsule, the inner aspects of the endplates develop no covering of tissue until a late stage when the fluid is finally replaced by a uniform tissue mass throughout the cavity   Secondly, the tissue distribution round the frame clearly conforms to the pattern of local cavities rather than to a simple envelopment of the perspex   Thirdly, in two control experiments, perspex plates curved to follow the outline of the subjacent tissues have been inserted and ligatured in a subcutaneous position corresponding to that of the frames   These plates are found only to be surrounded by thin capsules even though left in place for long periods, and no other tissue mass develops round them   The reaction to a foreign body

theiefore, while probably responsible for the capsule surrounding the frame, does not adequately account for the further growth of tissue filling either the cavity of the frame or the local cavities round it

Composition of tissue and formation of adipose tissue In histological structure and chemical composition, the tissue first formed appears to be fibrous tissue, containing only traces of fat (Table I) In tissue removed after less than seven weeks, ether extract has formed less than 2% of the total weight, water forming 89% of the remainder In two animals killed after 54 and 67 days, small but probably significant amounts of fat were found, ether extract comprising 15 and 3%, and water 85% of the remainder At this stage free fluid was still present below the frame, but in small amounts and with a low protein content In three animals killed after eight weeks or more, fat has been found in substantial amounts, forming an average of about 50% of the tissue mass Water has formed 79% of the remainder of the tissue In only one of these animals was free fluid found

Histologically, in material removed after less than eight weeks, adipose tissue appears sparsely, often as short chains of cells containing fat globules which commonly run parallel, and near, to blood vessels (Fig 5) Material removed later than eight weeks has contained masses of apparently normal adipose tissue with closely packed fat globules staining with Sudan III (Fig 6)

Since the adipose tissue develops in this way within a mass of initially fat-free fibrous tissue, it cannot be regarded as having been merely displaced into the cavity of the frame from surrounding areas Indeed, the frame is introduced into a space containing only occasional strands of areolar tissue and no visible adipose tissue The overlying subcutaneous tissue remains intact and freely mobile over the frame, and its fascia is uninterrupted and glistening when the frame is removed, so that the cavity does not appear to communicate with the subcutaneous adipose tissues Similarly the dorsal fascia deep to the frame is uninterrupted except at the site of the two ligatures and where vessels course over it to vascularise the contents of the cavity Little fat is, in any case, associated with this fascia Dorsal fascia sheets, equal in area to that of the base of the frame, have been found to contain only 0 1 g of ether extract or less (Table II) The capsule surrounding the control perspex plates has also been examined for fat and also contained only about 0 1 g of ether extract (Table II) Evidently, therefore, the adipose tissue masses found in the cavity of the frame result from growth and deposition rather than from displacement of adjacent tissues

In a few animals, the fluid contents of the frame cavity have been removed daily after insertion of the frame At first, fluid with a high protein content reforms within a day of each removal After about a week, however, the

TABLE 11

Control data

Rabbit Number	Tissue	Ether extract
		grams
5	Surrounding plate	0 11
7	,, ,,	0 07
5	Dorsal fascia	0 08
7	,, ,,	0 06
16	,, ,,	0 10

cavity becomes filled with tissue and fluid can no longer be obtained   In two animals so treated and killed at 20 days, 7 2 and 7 4 g of tissue had formed   This mass was greater than was found in unaspirated animals which at this stage had formed no tissue within the cavity and only formed smaller amounts later after insertion of the frame   It seems possible, therefore, that a cavity is more rapidly filled by solid tissue if its fluid content is repeatedly removed

DISCUSSION

One of several physical mechanisms might be responsible for initiating the sequence of tissue changes that occur within a cavity of the type described   In the first hours after the frame is introduced, the hydrostatic pressure within its cavity may be below that of the surrounding areas   This is clearly the case if air is withdrawn at the time of operation, when the overlying skin remains somewhat drawn into the cavity for hours or days   Once fluid has formed, this pressure difference must be much reduced or abolished   Presumably then the pressure in the cavity will approach capillary pressure in so far as the osmotic pressure of the cavity fluid approaches that of plasma , and fluid will be drawn from the capillaries into the cavity until this equilibrium is reached   The elasticity of the skin may still, however, reduce the tissue pressure locally in certain regions   Where the skin is drawn down into an angle between the frame and the back, instead of sweeping more directly from the summit of the frame to an adjacent area of back, the skin elasticity may tend to shorten this skin-fold and hence have a " suction " effect on the underlying tissues in this angle   The distribution of new tissue in the angles or subsidiary cavities surrounding the frame would be consistent with a mechanism of this sort , but it is hard to believe that the reductions in tissue pressure so produced would be large   Further work is required on this point and on the factors influencing fat deposition   The information available on this subject has been discussed by Cameron and Seneviratne (1) who emphasise the lack of quantitative information on growth and repair

of adipose tissue The present experiments may offer a method for closer study of some aspects of adipose tissue development and the local factors which initiate it

Whatever the physical mechanism involved, adipose tissue may be deposited to fill cavities created within certain parts of the body It is clearly possible that, by a similar process, fat may be progressively laid down to replace a slowly wasting structure or to fill a slowly increasing space In situations where adjacent structures cannot occupy the space, fat deposition may thus prevent a cavity from developing The replacement of atrophic bone marrow by fat may be a comparable process and other possible examples require investigation In particular, it might be anticipated that, if the eye prolapsed forward progressively from weakness of the orbital muscles adipose tissue would fill the increased retrobulbar space Such an explanation for the orbital findings in simple thyrotoxic exophthalmos evidently deserves examination therefore, but is clearly not established by the present findings and has indeed serious objections on other grounds

The local distribution of subcutaneous fat in man resembles in certain respects that of the adipose tissue forming around the perspex frame in the rabbit On the antero-lateral aspect of the upper arm, the subcutaneous fat as estimated by skin-fold measurements, is thin over the bellies of the biceps and triceps muscles Between these muscles, however, the fat thickness increases considerably, and a pad of fat occurs constantly in this situation which is about twice as thick as the layer over the muscle bellies Similar local thickenings may be found filling in the angle between the lower border of gastrocnemius and soleus, or surrounding but not covering prominent bony points The smoothing or 'rounding off' of the skin contour so produced is a familiar anatomical feature It is possible that local conditions in the sulci between prominent muscles or structures are similar to those in the angles or subsidiary cavities around the frame In each situation fat is deposited in such a way that the overlying skin is drawn less closely down into the angle between the subjacent tissues, and passes more directly from crest to crest of adjacent prominent structures It seems likely that the same local factors which cause deposition of adipose tissue in and around the frame may be responsible for the moulding of subcutaneous fat in man

SUMMARY

1 A small perspex frame has been introduced subcutaneously in the rabbit between the loose dorsal skin and the underlying muscle fascia The material filling the cavity within this frame has been examined at different times subsequently

2 The cavity is filled first with a clear fluid of high but gradually falling protein content (Fig 2)

3 After seven weeks, the fluid is being replaced by fibrous tissue (Table I)

4 After ten weeks, this tissue is being replaced by adipose tissue (Fig 6) which comes to form one third or more of the total tissue mass

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Fig 3
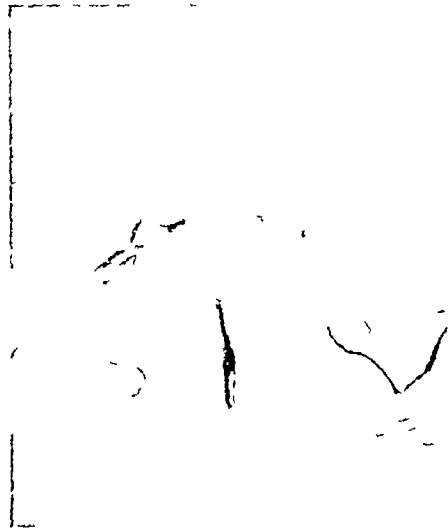
Fig 4

Fig 5
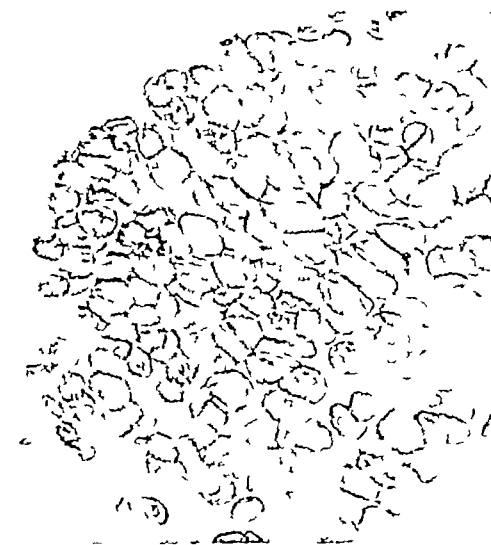
Fig 6

Fig 3   Tissue formed within the cavity of the frame, seen after reflection of skin and before removal of the frame (natural size)

Fig 4   Tissue mass after removal of frame (natural size)

Fig 5   Tissue from within frame in rabbit 6 after 67 days   Stained Sudan III, magnification × 85   Strands of small fat globules lie parallel to blood vessels in fibrous tissue

Fig 6   Tissue from within frame in rabbit 4 after 131 days   Stained Sudan III magnification × 85   Masses of large fat globules occur throughout the section

THE PLASMA IODIDE CLEARANCE RATE OF THE HUMAN THYROID *

By N B MYANT, E E POCHIN and the late E A G GOLDIE

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THE radioactive isotopes of iodine have been extensively used to follow the iodine metabolism of the body since they are metabolised in the same way as normal iodine, and since the distribution of a test dose can be followed and measured by means of its radioactivity   Such measurements are highly sensitive and a dose can be used which is small enough to produce no detectible change in the normal iodine metabolism, which is therefore accurately sampled by means of the isotope   This technique has been widely used on animals and in the study of thyroid metabolism *in vitro*, and is of particular value in man, since the gamma radiations emitted by the isotope pass through the tissues and can be detected by a Geiger counter placed opposite the thyroid gland

Radioiodine was used in the study of human metabolism in 1939 by Hamilton and Soley (7) who had shown previously (6) that the absorption of oral radioiodide into the tissues could be followed by an external counter in man   In normal subjects, 66% of a dose was excreted in the urine within 24 hours   The subsequent excretion rate fell exponentially with a half period of about 9 hours   Excretion was more prolonged in cases of myxœdema   The same authors in 1940 (8) used the isotope to follow the course of iodine uptake by the thyroid, using a counter placed on the skin over the thyroid isthmus, and found differences in the general course of the uptake curve in thyrotoxic, normal and hypothyroid subjects   The radioiodine was given with 14 mg of normal iodine as " carrier "   This work was extended in 1942 by Hamilton, Soley, Reilly and Eichorn (10) in a study of hypothyroid and normal children, using doses with a low content of normal iodine   It was shown that the forms of the uptake curves were profoundly influenced if the carrier dose of normal iodide was high   Radio-autographs were used to study the histological distribution of the radioiodine within the gland

Later work has extended each use of radioiodine in the study of human metabolism   by radio-autography , by analysis of urinary excretion , and by measuring the course of the thyroid uptake   Radio-autography has been applied particularly to identify areas of iodine concentration within glands removed at operation (5, 9, 15, 16) and gives detailed results if adequate doses of radioiodine are used   It offers, however, no measure of the rate of iodine metabolism by the thyroid   A study of the urinary excretion of a test dose has been more fruitful in the analysis of thyroid activity   The percentage of the administered radioiodine excreted within one or more days has been repeatedly determined both in tracer experiments (11, 14, 22, 23, 24, 25, 26), and in the course of therapy with this isotope (3, 4, 12, 17) It has been shown that excretion is almost entirely in the urine, and its rate falls to a low value within a few days of the test dose   Keating, Power, Berkson and Haines (14) have made a valuable and illuminating study of the time course of urinary excretion, and have introduced useful quantitative measures that have not been examined in other work on this subject   The proportion of the dose excreted initially is usually greater in normal subjects than in thyrotoxic patients, but figures from these groups overlap, so that a clinical assessment of thyroid function cannot be based reliably on these data   In addition, deductions as to thyroid function from urinary analysis are necessarily indirect, and the collection of all urine specimens is often unreliable

Several workers have developed the use of direct measurements of thyroid radioiodine uptake, using a counter opposite the gland (11, 12, 13, 21)   The general nature of the uptake curve in normal and thyrotoxic subjects has been clearly established in this way, although its course has not been fully or quantitatively studied   Astwood (27, 28, 29) has applied a useful analysis to the early phase of the uptake curve, particularly in the study of antithyroid and other drugs   Quantitative analysis of the thyroid uptake curve has however been restricted by the problem of calibration No adequate solution to this problem has been described despite the use of " phantoms " (21) or by counts on a subsequently excised gland (10, 22)

While studying the thyroid uptake rate in thyrotoxic and normal subjects, we have been impressed by the dependence of thyroid uptake and renal excretion rates upon plasma radioiodine concentration, and by the greater speed of uptake in the thyrotoxic subjects   We have therefore examined the quantitative relationships between these factors and their alteration in thyrotoxicosis, as already briefly noted (19)   The calibration of thyroid counting rate in terms of thyroid radioiodine content is described in a following paper (20)

Method

The radioactive iodine of atomic weight 131 has been used in these investigations   This isotope has a convenient half-period of radioactive decay of 8 0 days, so that its metabolism can be followed for a week or more

It emits both beta and gamma radiations   The former are sufficiently energetic to be counted without difficulty using robust counters and the radioiodine content of liquids may therefore be determined simply and rapidly   The latter have energies of 0 37 and 0 08 m e v and are transmitted through body tissues with little loss, so that the radioiodine contained in different parts of the body can be estimated using a counter at the body surface   Different counters have been used to estimate the beta radiation emitted from liquid samples, and the gamma radiation emitted from tissues within the body

Beta counting   Since the detection of beta radiation is much more efficient than that of gamma radiation, this method is used whenever samples containing radioiodine can be placed in direct contact with the counter tube   The activity of urine and plasma samples has therefore been determined in this way, and compared with that of standard solutions from which the test dose was taken   The liquid counter designed by Veall (31) has been used   This counter has a carbon cathode, is filled with an argon-alcohol mixture and is operated with an external quenching circuit   It has a glass wall which is surrounded by a glass sleeve   The space between counter and sleeve contains 10 ml of liquid as a thin layer round the counter and the count obtained depends largely on beta radiations   The particular counter used has had a background counting rate of 10 counts/min   when screened by lead and from light, and gave an additional count of 31 counts/min   for every 0 001 microcurie contained in the 10 ml of liquid necessary to fill the counter   Persisting contamination of the glass by active solutions was minimised by adding inert iodide as carrier to solutions counted, and with simple washing between determinations rarely caused trouble   The counter was operated at 1000 volts

Gamma counting   When determining the activity of body tissues which could not be placed in contact with the counter, the much more penetrating but less efficiently detected gamma radiations are used   The gamma counter used initially was a copper cathode argon-alcohol filled counter by M I T for which we are indebted to Dr Robley Evans   It was cylindrical, of diameter 3 0 cm and cathode length 10 5 cm and operated at 1280 volts   It had a background of 45 counts/min   as used, and gave an additional count of 7 8 counts/min   when its central wire was at a standard distance from a glass ampoule containing 1 microcurie of radioiodine   The standard distance was 51 5 cm , the ampoule axis then being 50 cm from the counter wall   Later work was done using a platinum cathode, argon-alcohol filled counter for which we are indebted to Mr Veall   The counter was cylindrical, 3 0 cm in diameter and of cathode 10 5 cm long, and operated at 1200 volts   This counter had a background of 47 counts/min   when screened from light, and gave an additional count of 17 counts/min   when 51 5 cm from a 1 microcurie source
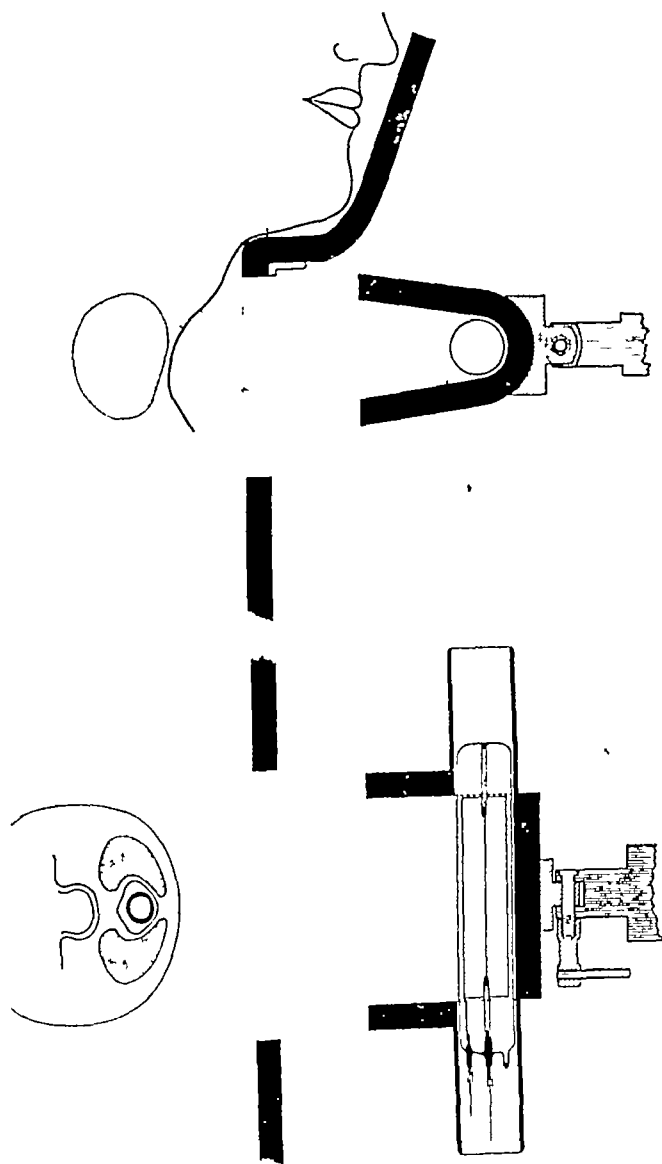
Fig 1   Plan view and sectional drawings of standard positions of counter, counter shield, lead screen and subject's neck   Lead in solid black, thyroid stippled   Scale, quarter natural size

Each gamma counter was used in a housing of lead $\frac{1}{2}$ inch thick and having a rectangular aperture $10\frac{1}{2}$ cm by 5 cm in a plane 6 cm from the axis of the counter (*see* Fig 1)   The counter was thereby largely screened from gamma radiations except over a solid angle 60° high by 105° wide As described later, the patient was also placed behind a lead screen to reduce radiations from areas not being studied (Figs 1, 7 and 8)

Procedure   Most of this work has been done using I^{131} from Oak Ridge, U S A which was obtained in carrier-free state   A few experiments were made using I^{131} from Chalk River, Canada, or from Harwell Supplies usually contained about 500 microcuries of I^{131}, carrier-free in 1 ml of distilled water, and were received in sealed ampoules, the activity of which had previously been estimated in terms of Oak Ridge values to which basis activities are referred in this paper *   The unopened supply was first counted with the gamma counter 51 5 cm from it so that the counting rate at this distance could be related to activity in microcuries, on the basis of the previous standardisation   A small amount of normal potassium iodide was added as carrier immediately the supply ampoule was opened, 40 micrograms usually being added to each 500 microcuries of I^{131} In making up a dose, a suitable fraction of the diluted stock solution was then made up to 50 ml in a measuring flask after adding further carrier to form a total of 20 micrograms in the flask   Of the contents, 0 2 ml were then withdrawn and diluted to 50 ml to act as a standard for comparison of urine and plasma samples in the liquid counter   The remainder was counted at 51 5 cm from the gamma counter and was then used as the dose

The dose was given by mouth to a subject who had had breakfast two or more hours previously, and was washed down by one or two small drinks of water   A dose of under 50 microcuries was used in all cases except two patients under investigation for retrosternal goitre or iodine-concentrating carcinoma of the thyroid   In all recent cases we have used the platinum cathode gamma counter and a dose of between 25 and 30 microcuries has been found adequate to give accurate data   The amount of iodide in the radioactive form is very small, having a mass of less than one thousandth of a microgram   It is safe to assume that the dose would not in itself modify thyroid behaviour either by its radioactivity or by its iodide content and would therefore sample the behaviour of an undisturbed thyroid gland As a precaution against disturbance by a high iodine intake, fish and medicines containing iodine were forbidden for two or more days before, and during the test

Within a few minutes of giving the dose, and at suitable intervals thereafter, a gamma radiation count was made opposite the thyroid   In all cases studied in detail, the subject sat with the chest against a lead shield

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* It appears likely that true activities are higher by about 70% than those given in the Oak Ridge consignment notices

which extended upwards from about the height of the xiphisternum to end in a chin rest, and extended laterally to screen the whole width of the chest (Figs 7 and 8)   The neck was placed so that the thyroid was opposite a rectangular aperture in the shield, and the counter was put in position opposite this aperture (Fig 1)   By this shield and by the local lead housing surrounding it, the counter was exposed to radiation from the thyroid, but protected from most of that from the remainder of the body   The counter would receive radiation from any radioiodine in the neck tissues surrounding the thyroid over a vertical extent of about 15 cm and horizontally over the whole width of the neck   The use of this shield also simplified accurate repetitions of the subject's position in successive readings   A marked point on the skin over the cricoid cartilage (Fig 8) was always brought to the same measured position behind the screen face and relative to the sides of the aperture   The seat height and the position of a posterior neck rest (Fig 7) were adjusted to known settings, so that the subject's general position was also controlled   The counter stand was rigidly linked to the lead screen, and the counter was mounted on a calibrated horizontal slide, so that its position

TABLE I

Variability of repeated estimates of thyroid counting rate

Subject	Percentage coefficient of variation		Calculated positional variation (mm)
	Observed	Expected from no of counts	
Thyrotoxic	1 37	1 50	—
Thyrotoxic	1 77	1 58	0 8
Control	1 74	1 90	—
Control	4 10	2 40	3 3
Thyrotoxic	2 00	1 25	1 5
Thyrotoxic	3 20	2 30	2 2

The variation in neck position is given as a standard deviation calculated from the difference between the variance of the counting rate observed and that predicted by the number of counts recorded in each determination

relative to the neck could be adjusted and varied accurately   Table I indicates that by this method successive measurements of counting rate could be obtained with a variability of about 2% from their mean value This figure is the standard deviation of counts on successive occasions in subjects in whom the counting rate was constant or only falling slowly Allowing for the statistical errors of counting, neck position can have varied

by only a few millimetres during the counts   In certain subjects, the lead screen was not used, and the front of the lead shield surrounding the counter was brought to a marked position on the patient's neck for each measurement   As will be seen later, the uptake curve can be followed adequately in this way, but the absolute amount of radioiodine in the gland can be estimated less accurately

Thyroid activity was usually measured every 5 or 10 minutes for the first two hours after the dose, then at about hourly intervals for the next four to six hours, then once or twice daily   Duplicate readings were made for all later counts, each being continued to a total of at least 500 and usually 2,000 or more counts   Measurements were also obtained in certain subjects, at initially frequent intervals, of the gamma radiation measured at a marked position over the thigh   This count was used to indicate the course of tissue radioiodide uptake and hence the approximate amount of radiation that would have been obtained from the exposed area of neck alone had the thyroid not been present   In a totally thyroidectomised subject the neck count was about half the thigh count   In all thyrotoxic and many normal subjects, thigh counts were low compared with counts opposite the thyroid , but in some normal and in hypothyroid subjects it is likely that radioiodine in neck tissues causes a substantial proportion of the count opposite the thyroid, particularly in the first hour after giving the dose   The thyroid radioiodine content can be expressed, as described elsewhere (20), as a percentage of the dose given, by comparing the counting rate opposite the gland with that observed when the counter had been exposed to the flask containing the dose before its administration, appropriate corrections being made for decay and for back-scattered radiations

The subject emptied his bladder just before the dose was given   For the first 1 to 3 days thereafter, each volume of urine passed was measured and timed, and the radioactivity of a sample of it counted   Subsequent 24 hours' excretions were pooled, measured and counted   All urine was collected in stoppered bottles containing 10 mgms of potassium iodide as carrier to minimise losses of radioiodine in handling   Ten millilitre samples were poured into the space surrounding the liquid counter   For early specimens after the test dose, a known dilution of urine was used since then the counting rate for undiluted urine ordinarily exceeded the limit of about 5,000 counts per minute above which counting rate ceases to be proportional to radioiodine concentration   In cases studied in detail, half-hourly specimens were obtained initially

Blood samples usually of 15 ml , were taken into 0 3 ml of a solution of 20% potassium oxalate and 4% potassium iodide as carrier   After centrifuging, the volume of plasma was measured, brought up to 10 ml if necessary, and counted in the liquid counter   After counting any group of urine or plasma samples, a sample from the 50 ml standard solution prepared

from the dose for that subject was also counted   The radioiodine concentration of plasma and urine could then be expressed as a percentage of the original dose contained in each ml of the sample

To determine the proportion of plasma radioiodine present as thyroxine, a modification of the method of Taurog and Chaikoff (30) was used   Thus 2 ml of plasma were shaken with 10 ml of butanol which were then twice extracted with 10 ml of 4N caustic soda and 5% sodium carbonate   The alkaline and the butanol fractions were counted in the liquid counter, correction being made for the initial 5-fold dilutions and for volume changes due to partial solubility of butanol and water   Where a more accurate estimate of the thyroxine radioiodine was required, 10 ml of plasma were extracted and the 50 ml of butanol brought down to 10 ml by evaporation

Results

The counting rate opposite the thyroid, or " thyroid count," begins to rise within about 3 minutes of oral administration of the dose to a fasting subject   In one subject who had breakfasted half an hour previously, the initial phase of the uptake curve was sigmoid only achieving its maximum rate of rise after half an hour   In all other cases the rate of rise of the thyroid count has been maximum within a short period of the dose, falling progressively thereafter   It will be appreciated that the counting rate is proportional to the radioiodine content of the thyroid, so the rate of rise of this count corresponds to the rate at which iodine atoms are accumulating in the gland

Normal subjects   In subjects without thyroid disease, the count rises steadily during the first hours usually reaching its maximum value after two to three days   In some cases the count then falls, but in others the maximum value is maintained on a " plateau " for many days before the fall starts   In one case, the count as corrected for radioactive decay was still on this plateau after six weeks when counting was inaccurate owing to decay of the isotope

The mathematical form of the uptake curve is discussed later in the light of the changes in plasma concentration   Stanley and Astwood's approximation (28) that the counting rate initially rises linearly with the square root of the elapsed time since the dose, is clearly inapplicable when the count is followed to its maximum   At the same time, it is useful to characterise individual curves, particularly in their speed of ascent   We have adopted, as a rough measure of rate of equilibration, the time since the dose until the counting rate reaches half the maximum value finally attained   We have termed this the " time of half-uptake "

The initial course of urinary excretion is similar to that of thyroid uptake in any one subject—the rate being rapid at first, but falling to a low value within 36 to 48 hours   In certain subjects, urine specimens were

obtained half-hourly for the first eight hours   The mean rates of excretion
over these intervals when plotted as percentages of the dose excreted per
minute, are found to decrease at first in an approximately exponential way
The time interval in which the excretion rate becomes halved is usually in
approximate agreement with the time of half-uptake of the thyroid count
in the same subject   Where repeated plasma samples have been taken,
the plasma radioiodine concentration at one hour after the dose has become
halved after a similar time interval (Fig 3 and Table III)   After 24 hours
the rapid fall in urinary excretion rate is replaced by an approximately
constant rate of excretion as observed by Keating and others in their study
of the total excretion (14)   The form of the curve of urinary excretion with
time is considered later in relation to the plasma concentration curve
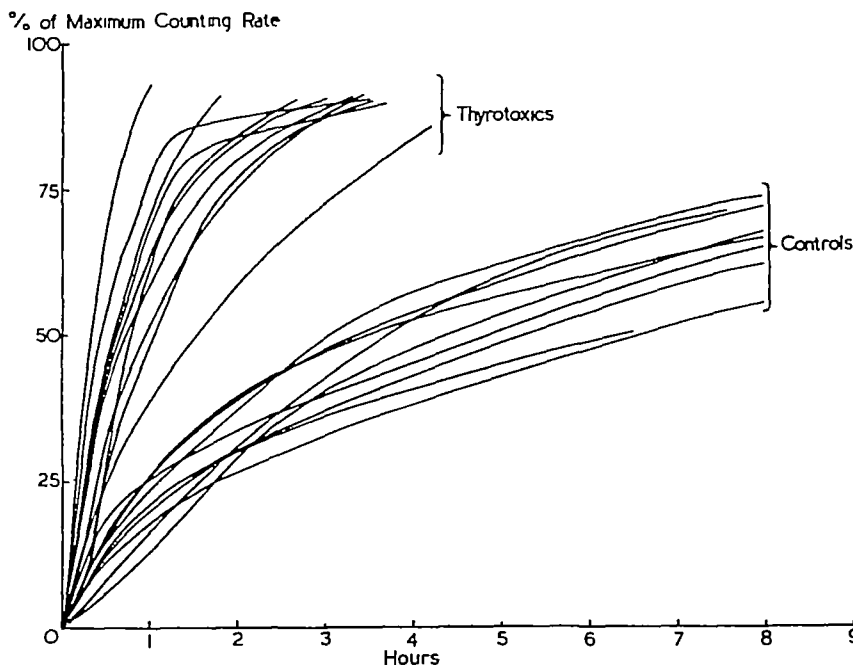
Fig 2   Thyroid uptake curves in thyrotoxic and control subjects—each curve in percentages
of the maximum counting rate reached in the subject plotted against time since an oral
dose   Curves from nontoxic goitres are not plotted, but are intermediate in position and
overlap both groups

Thyrotoxic subjects   In untreated thyrotoxic subjects, the thyroid
count begins to rise rapidly within a few minutes of ingestion of the dose
It rises ordinarily to a higher maximum value, and reaches this value earlier,
than in normal subjects   The maximum counting rate has averaged three
times the mean value for normal subjects, and has been reached after an
average time of 10 hours   The counting rate has then in some cases been
maintained as a plateau for several days, but more usually begins to fall

within 24 hours of the dose   The rapid attainment of the maximum counting rate in thyrotoxicosis is reflected in a shorter time of half-uptake and this time can be more accurately estimated than that of the maximum counting rate   Thyroid uptake curves for thyrotoxic and normal subjects are plotted in Fig 2 in terms of the maximum counts reached   It will be seen that half-uptake is usually reached between ¼ and 1¼ hours in thyrotoxicosis as compared with 3 to 6 hours in subjects with normal thyroids   The contrast in actual rates of uptake is even more pronounced since the maximum value attained is itself greater in thyrotoxicosis
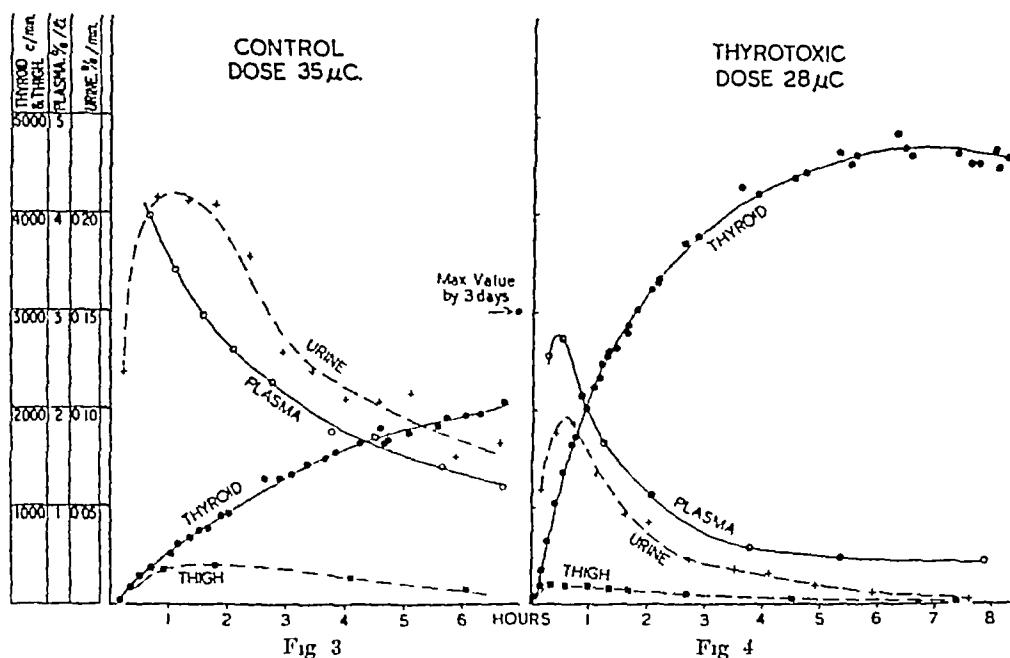
Fig 3   Curves of counting rate opposite thyroid and thigh , plasma radioiodine concentration as % of dose given, per litre , and rate of urinary radioiodine excretion, as % of dose given, passed per minute   Values plotted against time in a typical normal subject given 35 μC radioiodide by mouth at zero time

Fig 4   Curves as in Fig 3, but in a typical thyrotoxic subject given 28 μC

The rate of urinary excretion of radioiodine in thyrotoxic subjects has decreased more rapidly than in controls   When half-hourly specimens have been obtained, the rate of output has been found to fall in an approximately exponential way, and with time characteristics corresponding to those of the thyroid uptake curve in the same patient and of the plasma radioiodine concentration curve when determined (Fig 4, Table III)   After a few hours the output rate becomes roughly constant, and at a value usually substantially higher than in normal subjects   The smaller urinary excretion of radioiodine within 24 hours of the dose that has frequently been reported, is illustrated in Table III

Comparison between normal and thyrotoxic subjects   The transport of iodide in thyrotoxic subjects therefore differs from that in controls in two main ways   Firstly, the thyroid initially secures a greater, and the kidneys a smaller, share of the total dose than in control subjects   Secondly both processes, thyroid uptake and renal excretion, approach completion in a shorter time than in control subjects   These effects are explained since the thyroid is removing iodide from the plasma at an increased rate in thyrotoxicosis while the renal removal rate is unchanged   As a consequence of the more rapid removal of iodide by the thyroid, the plasma becomes depleted of radioiodide sooner, and so the rates at which it is removed by both thyroid and kidneys fall to zero earlier

The following hypothesis has been examined and appears to account for the observations   It is suggested that an approximately constant fraction of the total plasma iodide is removed each minute by the thyroid, or, as this may be more conveniently expressed, that a certain volume of plasma is cleared of its iodide each minute   As with the conventional renal clearances of urea and other substances, the thyroid clearance of iodide from the plasma can be readily determined if the plasma concentration of radioiodide is known at a moment when the rate at which radioiodide is entering the thyroid is also known   If for example, the plasma contains 1% of the dose of radioiodine, as iodide, per litre, and the rising curve of the thyroid count indicates that the thyroid is accumulating iodide at a rate of 1% of the dose per hour, the equivalent of one litre of plasma per hour would be being cleared of iodide by the thyroid   As with the renal urea clearance, it is not assumed that this volume of plasma is in fact fully cleared of iodide   If the efficiency of clearance by the thyroid were 25%, the actual plasma flow through the thyroid would be 4 litres per hour in this case   Further, since radioiodide is metabolised as normal iodide, the clearance rate determined by its use would be that applying to plasma iodide as a whole   The variability of the thyroid clearance rate with time or with total plasma iodide concentration is discussed later   The renal plasma clearance rate for iodide can similarly be determined by comparing plasma concentrations and rates of urinary excretion of radioiodide

Determination of clearance rates   Experiments have therefore been designed to test whether a constant volume of plasma is cleared of its radioiodide content by the thyroid and kidneys each minute   The dose given will not in itself be likely to alter the previous iodine metabolism in any way, since the amount of iodide administered is small   It should therefore merely allow the normal rates of plasma clearance to be sampled, and the values of these clearances should not be dependent on the time since the dose was given   If however, the administered radioiodine ceased to be fully available to the thyroid or kidney as iodide, or if the thyroid uptake rate were masked by an early and concurrent output from the thyroid, the

value obtained for the clearance might only be valid shortly after administration of the dose, and the time of its determination might be critical

We have examined the data in patients from whom repeated plasma samples were taken during the course of thyroid uptake and renal excretion of an oral dose of radioiodine   Typical results in a control and in a thyrotoxic subject are given in Figs 3 and 4 respectively   Each figure demonstrates the falling plasma concentration of radioiodine ,   the
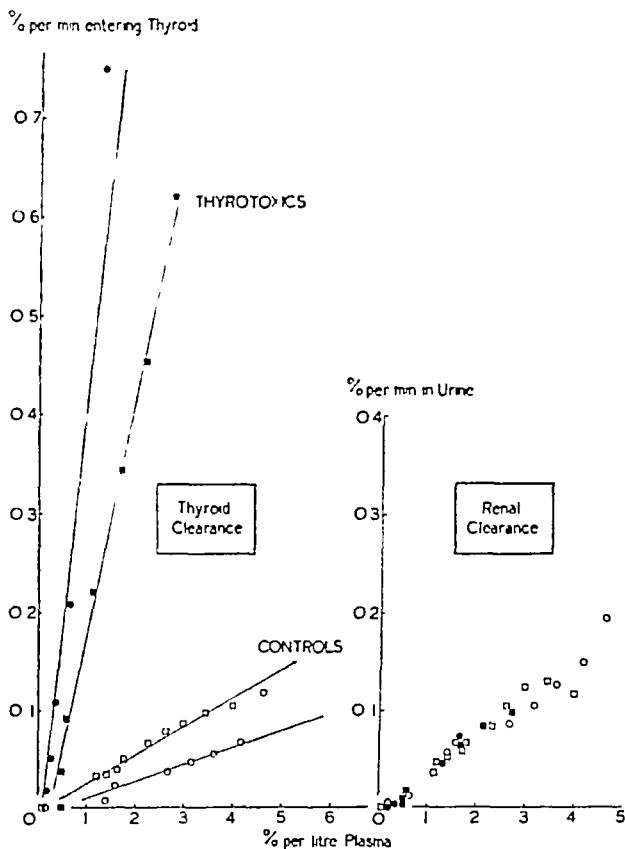
Fig 5   Fig 6

Fig 5   Graphs relating the rate of accumulation of radioiodine in the thyroid (in % of the dose per minute) to the simultaneous value of its plasma concentration (in % of the dose per litre)   Data for two thyrotoxic (solid squares and discs) and two control subjects (open squares and circles)

Fig 6   Graphs relating the rate of urinary excretion of radioiodine (in % of the dose per minute) to the simultaneous value of the plasma concentration (in % of the dose per litre)   Symbols as in Fig 5

corresponding fall in the rate of its urinary excretion , and the thyroid count rising at a rate which also decreases as the plasma concentration falls   It will be seen that in the thyrotoxic subject (Fig 4) each change approaches completion earlier.

Direct comparison between thyroid uptake rates and the simultaneous values of plasma concentrations are illustrated in Fig 5 , and those between renal excretion rates and plasma concentration in Fig 6   Each figure presents the results on two thyrotoxic and two control subjects, based on repeated plasma samples obtained during the eight hours after an oral dose of radioiodide   It will be seen from Fig 5 that the rate at which radioiodine is being taken up by the thyroid remains roughly proportional to its plasma concentration during these hours, the thyroid clearance rate therefore being about constant over this period   In the two thyrotoxic subjects, however, the lines relating these variates are steeper than in the controls, indicating that the thyroid clearance rates are increased   The rates of renal excretion also remain proportional to plasma concentrations over this period (Fig 6) In this case the lines for all subjects have equal slopes, indicating that the renal clearance rate for radioiodide is not increased in the thyrotoxic subjects

The values of the clearance rates, estimated at different times in these four subjects, are shown in Table II   Estimates made between one and six hours after the test dose agreed closely in the normal subjects, the coefficients of variation of thyroid and renal clearance rates averaging 8% Earlier estimates of thyroid clearance made within an hour of the dose have been unreliable in normal subjects, probably because during this period the body tissues are still taking up radioiodide   Uptake by neck muscles therefore contributes significantly to the rate of rise of the counting rate opposite the thyroid   After an hour, the general tissue content of radioiodine changes only slowly, as is shown by the course of the thigh count (Fig 3)   The rise of the counting rate opposite the thyroid then measures thyroid uptake of radioiodide and the clearance rate can be reliably estimated from it

Early estimates of thyroid clearance are less liable to such errors in thyrotoxic subjects   In them the thigh count is low relative to the thyroid count (Fig 4), indicating that changes in the neck tissues are unlikely to influence the value obtained for the thyroid clearance rate   Late estimates of thyroid clearance, on the other hand, are more liable to error in thyrotoxic subjects, owing to earlier discharge of radioactive thyroxine from the gland The radioiodine content of the plasma then ceases to measure radioiodide only, and iodide clearance rates can no longer be based upon it   In addition, changes in the thyroid counting rate are due to the difference between the uptake and output of radioiodine by the gland   For both these reasons, the thyroid clearance may be increasingly underestimated after the initial hours in thyrotoxicosis, and in some cases cannot be accurately determined in consequence (Table II, thyrotoxic subject 2)   Similarly, the line relating the rate of rise of the thyroid count to the plasma radioactivity does not pass through the origin of the graph , but shows that the thyroid count ceases to rise although the plasma is still radioactive   Even in normal subjects, this

TABLE II

Values of clearance rates (ml/min) in two thyrotoxic and two control subjects as estimated at different intervals after a dose of radioiodide

Controls

	Subject 1			Subject 2		
Time hr	Clearance Thyroid	Renal	Time hr	Clearance Thyroid	Renal	
0 6	26	30	0 8	26	42	
1 1	28	38	1 5	16	36	
1 6	29	42	2 2	15	35	
2 1	30	40	3 0	15	33	
2 8	29	37	4 0	14	37	
3 8	20	38	5 4	14	45	
4 5	25	35	6 7	56	40	
5 6	24	37				
6 6	28	39				
Mean (1 to 6 hr)	28	38		15	37	
Coeff of var %	8	6		6	12	

Thyrotoxics

	Subject 1			Subject 2		
Time hr	Clearance Thyroid	Renal	Time hr	Clearance Thyroid	Renal	
0 2	361	31	0 5	665	44	
0 5	227	36	1 0	580	36	
0 8	211	39	1 6	330	38	
1 2	206	39	2 2	300	39	
2 0	196	32				
3 8	158	26	3 7	185	19	
5 3	77	15	4 9	92	11	
7 8	0	7	5 9	0	5	
			7 6	0	4	
Mean (½ to 3 hr)	210	36		470	30	
	6	0		26	9	

lme does not pass exactly through the origin and some discharge of radioiodine from the thyroid probably occurs at this period   These differences are consistent with the changes observed in plasma radioactivity   In thyrotoxic subjects, an initial fall due to decreasing radioiodide concentration is succeeded by an early and considerable rise due to radiothyroxine development   In one case radiothyroxine was detectable in the plasma 3 hours after the dose   In normal subjects this secondary rise occurs later and reaches a lower concentration, and radiothyroxine has not been found within 2 days of the dose

It appears therefore that the thyroid clearance rate may be reliably determined between the time at which the thigh count reaches a maximum and that at which radioactive thyroxine is detectable in the plasma   Estimates made an hour after the test dose are likely to be reliable in most subjects, but we have felt it desirable to determine clearances on several plasma samples taken over the first few hours following the dose   The determination is clearly not adapted for routine use in a standardised form, although its consistency in normals (Table II) and its considerable elevation in thyrotoxicosis may make even an approximate estimate useful   It will be appreciated that no error is introduced if, for example, the dose is only partially absorbed, since the thyroid clearance rate depends only on the relationship between thyroid uptake rate and the simultaneous plasma concentration, however attained

The renal clearance rate may be determined within the same time limits as for the thyroid clearance rate   Early estimates clearly may involve errors due to delay in urine formation or collection   Late estimates when the plasma contains radioactive thyroxine have been found to involve discrepancies similar to those in the corresponding thyroid clearance values   Reasonably constant estimates of renal iodide clearance have been obtained with samples taken between one and six hours after the dose (Fig 6 and Table III)

Clearance rates in normal and thyrotoxic subjects   The values of the renal clearance rates given in Table II are directly obtained by comparing the plasma and urinary concentrations of radioiodine and recording the rate of excretion of urine   Since these concentrations are proportional to counting rates in the same counter, no difficulty in calibration is involved   The absolute value of the thyroid clearance is harder to determine accurately, and this problem is considered more fully elsewhere (20)   Plasma concentration can readily be expressed in terms of the dose , and thyroid uptake rate can be given accurately in terms of counting rate under specified conditions   The counting rate due to radioiodine in the thyroid must then be related to that which would be caused by the whole dose   In the 12 subjects studied in detail, this has been carried out by a relatively accurate method (20) involving allowance for back-scattered radiations and using counts at different distances from the neck to determine the position and

TABLE III

Clearance and other rates in thyrotoxic and control subjects

| Age | Sex | B.M.R. (%) | Clearance rates | | Urinary 24 hr excretion (% dose) | Half times of — | | | Time of max thyroid uptake (hr) |
			Thyroid (ml/min)	Renal (ml/min)		Plasma conc (hr)	Renal rate (hr)	Thyroid count (hr)	
Controls									
57	F	+19	8*	11	51.4	—	7.0	6.3	48
50	F	+14	21*	24	53.8	—	5.9	3.3	36
62	M	—	—	—	59.8	—	4.7	5.0	120
36	M	—	—	—	82.4	—	—	0.0	45
57	F	-6	0*	—	61.1	—	5.4	3.0	24
27	F	-5	12	44	64.2	+4.3	4.8	5.2	72
29	F	+20	10	42	71.7	7.0	4.7	—	40
80	M	—	15	31	54.2	3.7	7.0	0.5	56
47	M	—	38	37	64.3	2.8	3.7	3.3	48
22	M	—	18	28	47.9	—	3.8	3.7	72
21	F	+14	—	—	—	—	—	4.5	—
		Mean	16	31	60.7	(4.4)	5.2	4.7	56
Untreated thyrotoxic									
49	F	+49, 53	300*	31	11.2	—	1.2	1.5	10
29	F	+85, 84	1300*	46	2.4	—	1.3	0.4	8
39	F	+60, 62	930*	10	8.0	—	1.0	0.0	10
51	F	+102, 98	108*	25	5.1	—	(3.0)†	(2.2)†	7
26	F	+39, 30	199*	16	9.4	1.3	1.7	1.3	12
28	M	—	210	36	13.4	0.7	1.2	1.1	7
33	F	+77, 78	470	30	7.1	0.8	1.0	0.9	5
28	F	+46	300	30	9.2	1.1	1.3	0.6	15
20	F	+56, 55	088	19	9.8	0.4	1.0	0.6	19
33	F	—	273	37	5.5		3.3	0.7	10
28	F	+49, 47	326	12			1.6	0.7	5
		Mean	480	27	8.0	(0.8)	1.5	0.9	10

Goitre, with normal B M R

		B M R							
19	M	— 6	80*	—	41 0	—	0 5	11	12
33	F	+ 5	100*	—	—	—	—	16	13
32	F	—11	28	50	34†	2 0	—	23	24
00	F	+18	33‡	14	28 2	3 7	4 0	3 1	—

* Determined by approximate method

† Uptake delayed by recent meal

‡ Case of Hashimoto's thyroiditis

Half times were estimated as the duration in which —

(a) the plasma concentration at one hour became halved,

(b) the initial renal rate of excretion was halved,

(c) the thyroid count rose to half its final maximum value

strength of the thyroid radioactivity   In other subjects, approximate values were determined by calibrating the thyroid counting rate indirectly   At a time when the plasma concentration was low, the thyroid was assumed to contain all the dose except that already excreted in the urine, a small correction being made for radioiodide in the body   The results in all subjects are given in Table III with data on the course of thyroid uptake, renal excretion and plasma concentration of radioiodine

In the normal subjects examined, the thyroid iodide clearance has ranged from 8 to 38 ml of plasma per minute, with a mean value of 16 ml/min   In 11 cases of untreated thyrotoxicosis, its value has ranged from 200 to 1390 ml/min averaging 486 ml/min   The volume of plasma cleared of iodide per minute has thus been increased in thyrotoxicosis to a mean of 30 times its normal value   By contrast the renal clearance rate has averaged 31 ml/min in normal and 27 ml/min in the thyrotoxic subjects, the difference being insignificant statistically   Evidently, this greatly raised thyroid clearance and unchanged renal clearance in thyrotoxicosis underlie the greater and more rapid uptake of radioiodine by the thyroid, with reduced initial renal excretion

The normal renal clearance for iodide is about 23% that given for the inulin clearance in man by Homer Smith (25), and indicates that iodide is substantially reabsorbed in the renal tubules   The normal thyroid clearance cannot be directly interpreted, since data for human thyroid blood flow are uncertain   Best and Taylor (2) give an estimate of 3½ to 6 ml/min/gm, and Means (18) one of 4 ml/min/gm which, with a hæmatocrit of 45% and a 30 gm gland, would imply a thyroid plasma flow of 50 to 80 ml/min   On this basis an iodide clearance of 16 ml/min would indicate that the normal efficiency with which the thyroid removed iodide from plasma was between 20 and 30%   When thyroid clearance rates averaging 30 times the normal value are recorded in thyrotoxicosis, it is evident that the blood flow to the gland must be greatly increased, and it is likely also that iodide is removed with increased efficiency   In the case with the highest clearance recorded, the thyroid blood flow must have been 3 litres/min even if plasma iodide removal was completely efficient   This value already exceeds half the normal cardiac output, and it is difficult to believe that removal can have been much less than fully efficient   The gland in this patient was judged to be highly vascular, having a thrill and a bruit   It is evident that the increased blood flow through the gland may in such cases be an important contribution to the raised cardiac output observed in this condition

The thyroid clearance rate is a product of thyroid plasma flow and of the efficiency with which iodide is removed from this plasma   The clearance rate might therefore be increased if the thyroid were enlarged, even though its tissue were less vascular and less efficient at removing iodide   For example, if the plasma flow per gram of gland and the efficiency were both halved, but the gland were increased to eight times its normal size, the

clearance should be doubled   We have examined three patients with goitre who were admitted for investigation of thyrotoxicity, but had normal metabolic rates and no conclusive evidence of thyroid overactivity   The thyroid clearances were 28, 86 and 100 ml/min   the values in two cases therefore being abnormally raised but not to the extent associated with clear thyrotoxicosis   It is uncertain whether these should be regarded as cases of mild thyrotoxicosis or of simple goitre with moderate elevation of the thyroid clearance rate

Discussion

In all the thyrotoxic patients we have studied, the thyroid clearance rate has considerably exceeded the highest value observed in normal subjects   The clearance is calculated as the ratio between rate of rise of thyroid content and the corresponding plasma concentration of iodide and is thus derived from three factors, each of which is altered in thyrotoxicosis   Firstly, we have found the average maximum count in thyrotoxicosis to be about three times that in controls   Secondly, the time by which half this value is reached has averaged 0 9 hours in thyrotoxicosis as against 4 5 hours in controls   Thirdly, the plasma concentration in control subjects has been about 2 5 times that in thyrotoxic subjects at 1 hour after the dose   Since the thyroid iodide clearance is calculated from the product of three factors closely related to these, it forms an index which is more sensitive than any one of these factors taken singly

The relationship between different indices of thyroid function and their value in estimating over-activity is made clearer if the dynamics of iodine transport are considered   The following mathematical analysis relates the value of the thyroid clearance rate to certain less direct estimates of thyroid activity which have been obtained by the use of radioiodine

It is useful to discuss first the simple case in which diffusion of iodide through tissue spaces is assumed to be rapid and thyroxine liberation from the gland slow compared with thyroid and renal uptake of iodide   The latter assumption is approximately true in many patients whereas the former will require modification below   Suppose that shortly after administration the whole dose of iodide is uniformly distributed through an iodide space of volume V litres Suppose also that the thyroid clears a constant amount g and the kidneys clear r litres of plasma per minute of its contained iodide   If diffusion of iodide is assumed to be rapid the concentration c of radioiodide in the plasma is the same as that in the whole iodide space V and the value of V is constant   Now the fraction of diffused iodide removed per minute to the thyroid is $\frac{g}{V}$ and that to the urine is $\frac{r}{V}$ The total fraction of its iodide lost by the iodide space in time dt is therefore $\frac{g + r}{V}dt$ Further since the diffusion is assumed to be rapid this may be equated to the proportional fall in iodide concentration in the same time whence

$$-\frac{1}{c}\frac{dc}{dt} = \frac{g + r}{V} \qquad (1)$$

Since g r and V are constant the plasma concentration falls by a constant proportion of its value each minute   Such an exponential fall will be given by

$$C = C_0\, e^{-\frac{g + r}{V}t} \qquad (2)$$

where C_0 represents the initial concentration if the whole dose were distributed through the volume V

It can similarly be shown that, under these conditions, the amount x of radioiodine in the thyroid would rise *exponentially towards a plateau value,* so that

$$x = \frac{g}{g + r} \, C_0 V \left(1 - e^{-\frac{g + r}{V}t}\right) \qquad (3)$$

and the amount u passed in the urine by a time t would rise exponentially so that

$$u = \frac{r}{g + r} \, C_0 V \left(1 - e^{-\frac{g + r}{V}t}\right) \qquad (4)$$

It will be seen that all three processes are exponential with the same half time $T = \log_e 2 \cdot \frac{V}{g + r}$ which is inversely proportional to the sum of the thyroid and renal clearance rates

When equilibrium of these reactions is nearly attained, c, the plasma iodide concentration, has fallen to a low value, x, the radioiodide in the thyroid, has risen to $\frac{g}{g + r} \, C_0 V$, whereas u, the amount excreted in the urine, has risen to $\frac{r}{g + r} \, C_0 V$ It will be seen that tests depending on a direct determination of g are likely to be more sensitive than those depending on a determination of T or of the limiting fractions of the dose excreted in the urine or retained in the thyroid, these fractions being equal respectively to $\frac{r}{g + r}$ and $\frac{g}{g + r}$ Table IV shows the way in which these indices alter with changes in value of g, taking figures of 30 ml a minute for r, and of 20 litres for V, as discussed below It will be noted that increases in the value of g, particularly when moderate, are only poorly reflected in changes in the other three indices It is probably for reasons of this kind that the direct determination of the thyroid iodide clearance appears to be a more sensitive and direct measure of thyroid function than the other indices previously used which depend on functions involving the thyroid clearance rate less directly For the same reason, its values in normal and thyrotoxic subjects may prove to overlap less than those of other indices have been shown to do

The formulae given above are only approximations to the course of iodine transport, because the rate of iodide diffusion through the body spaces is not in fact a rapid one Barlow has recently studied the rate of entry of radioiodide into the cerebro spinal fluid from plasma in man and finds that equilibrium in concentrations is only approached gradually in the course of the first days following oral doses to normal subjects (1) It is clear, therefore, that the volume V through which the test dose is diffused is not in fact constant with time since the dose was given It is consistent with this that the curve of plasma radioiodide concentration does not always fall exponentially with time but at a speed progressively slower than the exponential curve would indicate, and in a way consistent with a progressively increasing iodide space For this reason, equation (1) is replaced by

$$-\frac{1}{c}\frac{dc}{dt} = \frac{(g + r) + \frac{dV}{dt}}{V} \qquad (5)$$

since the fraction of diffusible iodide cleared per minute must be equated to a change in the product cV of two terms each of which is varying *

The variation with time of the volume V is not directly relevant to the present observations, except as accounting for a departure from exponential form in the curves of plasma radioiodine concentration, and of thyroid and renal uptake Our observations are consistent with a volume which increases with time over some hours as the dose diffuses through the tissue fluids Accurate study of this subject would require information on initial distributions by intravenous doses which we have not used It has been noted however (Table III) that the time of half uptake averaged 0 9 hours in thyrotoxic and 4 5 hours in control subjects If such values are treated as if they were half periods of exponential uptake, and are related to the corresponding values of g and r in the formula for these half periods

$$T = \log_e 2 \ \frac{V}{g + r} \qquad (6)$$

a value of 20 litres is obtained for V in normal subjects and higher values in thyrotoxic patients

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* Since the expansion of the volume V itself contributes to a fall in plasma radioiodide concentrations, it appears that the ascription (14) of a "removal fraction" largely to thyroid removal of iodide may fail during this period

TABLE IV

Relation between thyroid clearance rate and other indices of thyroid function

		10	20	50	100	100	1000
Thyroid clearance rate g	ml/mn						
Time of half uptake T	hr	5 7	4 6	2 9	1 8	0 5	0 2
Renal excretion of dose $\dfrac{r}{g+r}$	%	75	60	38	23	7	3
Thyroid retention of dose $\dfrac{g}{g+r}$	%	25	40	62	77	03	97

Values calculated on the basis of formula (6) taking $r = 30$ ml/mn and $V = 20$ litres

If the value of V is in fact increasing, so that $\dfrac{dV}{dt}$ in equation (5) is positive, these will be underestimates of the iodide space. It seems reasonable to assume, therefore, that the radioiodide diffuses through a volume comparable with the body water after some hours. This conclusion is important, if correct, since the amount of diffused radioiodide may be substantial, and the thyroid content differ markedly from the amount unaccounted for by urinary excretion. The thyroid clearance rate, on the other hand, is independent of the course of any diffusion process

In subjects investigated in detail, the thyroid clearance rate has been approximately constant over the course of several hours. We have not examined its variation from day to day, or its dependence upon dietary iodine. Presumably the daily thyroxine output from the thyroid is not determined simply by the day's iodine intake. It is possible, therefore that the thyroid clearance rate varies from day to day to control the level of thyroxine output. It seems more likely, however, that other factors are responsible for such control since the thyroid clearance rates of normal subjects on normal diets have lain within a relatively narrow range. Several other factors might vary the rate of thyroxine synthesis, storage or release so as to maintain a steady thyroxine output despite a varying iodide intake by the gland. It seems likely that a study of radioiodine metabolism may clarify these factors involved in physiological control as well as those in pathological conditions. Meanwhile the thyroid clearance rate measures directly the activity of the thyroid in taking up iodine from the plasma. In so far as a constant proportion of all iodine taken up by the thyroid is put out as thyroxine, the clearance should also indicate the rate of thyroxine output. This correlation will, however, fail under thiouracil treatment, or when the thyroid iodine content is changing or if varying amounts of iodine are being liberated from the thyroid other than as thyroxine

SUMMARY

1. Thyroid clearance rates for radioactive iodine have been calculated on the same lines as the conventional renal clearance rates for other substances. If both the rate at which radioiodide is entering the thyroid, and the simultaneous plasma concentration of radioiodide are known, then the volume of plasma cleared of radioiodide in unit time can be calculated

2. In normal subjects about 16 ml of plasma are cleared of iodide per minute by the thyroid. In 11 untreated thyrotoxic patients, the clearance rate has averaged 486 ml per minute. Values ranged from 200 to 1400 ml per minute in individual subjects

3. The renal clearance rate for plasma radioiodide averaged about 30 ml per minute both in normal and in thyrotoxic subjects

4. The thyroid clearance appears to be a more sensitive and direct measure of the iodine-collecting activity of the thyroid than previous tests based on the use of radioiodine. It may prove to have a greater value than the basal metabolic rate in diagnosing minor degrees of untreated thyrotoxicosis and in investigating the mechanism of the disease

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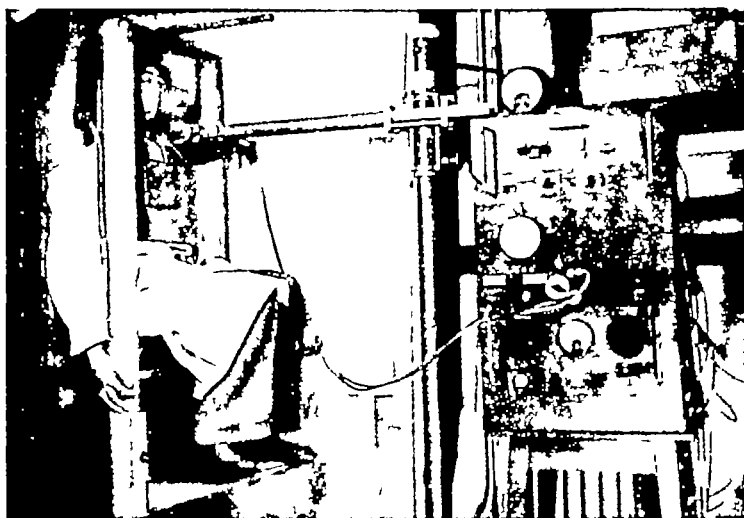(31) VEALL   Brit J Radiol 1948, **21**, 347

Fig 7

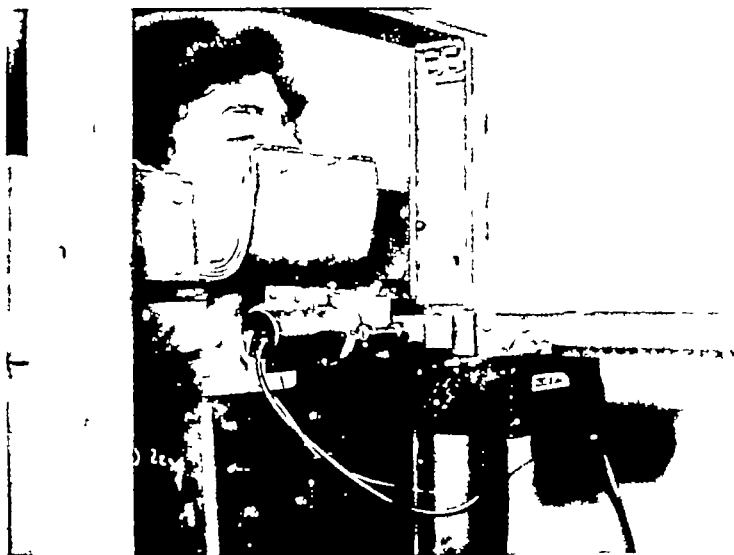

Fig 8

Fig 7   Arrangement of subject screen and counter ensuring constancy of subject's position relative to the counter and screening of the counter from extraneous radiation

Fig 8   Detail of relationship between counter and neck   The marked point on the neck is kept at a constant distance behind the lead screen, and the counter position adjusted by a horizontal scale reading

THE ESTIMATION OF RADIOIODINE IN THE THYROID GLAND OF LIVING SUBJECTS *

By N B MYANT, A J HONOUR and E E POCHIN

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If a subject is given a dose of radioactive iodine by mouth the presence of radioiodine in the thyroid is detectible within a few minutes by means of a Geiger counter placed in front of the neck   The rapid accumulation and slower discharge of radioiodine may then be followed from the rise and fall in the external counting rate   The shape of this uptake curve has been examined in detail by several workers but no one has described an adequate method of calibrating the external counting rate in terms of the radioiodine content of the thyroid   Methods previously described (including 2) appear to make insufficient allowance for the effects of thyroid position or back-scattered radiation

The radioiodine content of a sample suspended in air may be estimated by comparing the counting rate at a standard distance between sample and counter with the counting rate given by a sample of known strength at the same distance   Now the counting rate varies inversely as the square of the distance from the source over a range of distance depending on the size of the source and counter   Therefore, within wide limits, a counting rate at a given distance from the source may be expressed as a counting rate at the standard distance   Hence, it should be possible to estimate the radioiodine content of a thyroid from the counting rate obtained at a known distance We have used a method based on this principle , but for a thyroid within the body two modifications are required   Firstly, since the extent of the thyroid cannot be defined in the living subject its position must be found indirectly   Secondly a correction factor must be introduced because the neck tissues cause an apparent increase in the radioactivity of the thyroid by back-scatter of a proportion of the radiations

In this paper we give a method for estimating the position of the thyroid and describe experiments from which the amount of back-scatter can be determined approximately

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Estimation of the position of a thyroid gland in air

Method The position of a dissected thyroid gland injected with radioiodine and suspended in air was measured indirectly by the following method Counting rates are determined with the counter at successive
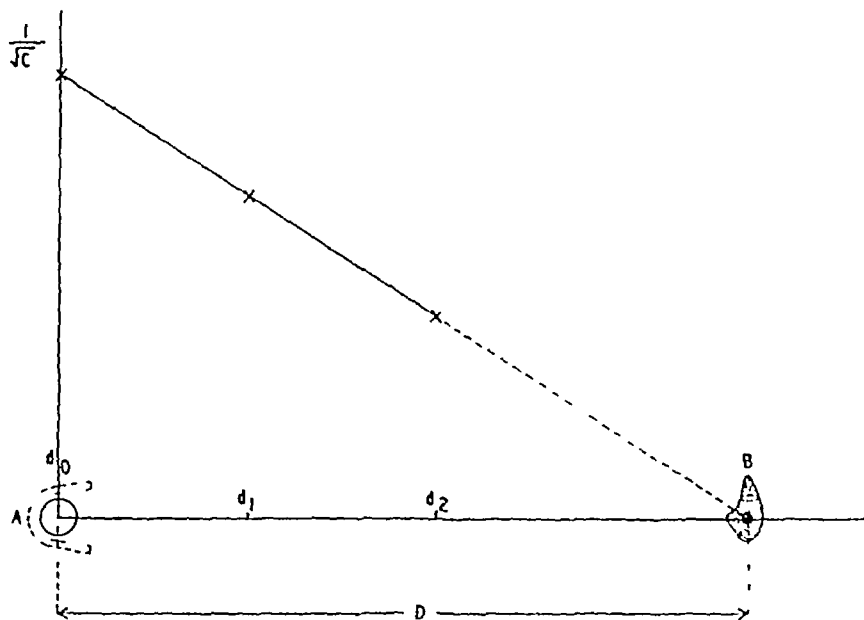

Fig 1 Diagram illustrating procedure for estimating D
   A   Cross section of shielded counter at zero position d_0
   B   Thyroid in profile
     The reciprocal of the square root of the counting rate C is plotted against the corresponding distances between counter and thyroid

distances d_1, d_2 etc from an arbitrary zero position at an unknown distance D from the thyroid Then, over the range in which the inverse square law is obeyed, C (the counting rate) is proportional to $\dfrac{1}{(D-d)^2}$, or D—d is proportional to $\dfrac{1}{\sqrt{C}}$ If, therefore, the values of $\dfrac{1}{\sqrt{C}}$ are plotted against the values of d, the points lie on a straight line which cuts the horizontal axis at a value of d corresponding to the centre of radiation of the source The procedure is illustrated in Fig 1 When the value of D has been so determined, the counting rate at the standard distance may be calculated from the value of $\dfrac{1}{\sqrt{C}}$ corresponding to this distance

Using this method, we estimated the position and radioactivity of thyroids each injected diffusely with a known quantity of radioactive iodine

Results The results are given in Table I

TABLE I

% error in estimating radioiodine	Distance between zero position and —		Difference (Dr — Dg) in cm
	geometric centre (Dg) in cm	calculated centre of radiation (Dr) in cm	
+2 7	53 2	53 1	—0 1
—3 5	64 5	64 8	+0 3
—2 3	59 0	59 0	0 0
+4 5	68 9	67 5	—1 4
— (not estimated)	68 9	68 2	—0 7

In all cases, the error in estimating the amount of radioiodine was less than 5% and the predicted centre of radiation lay at a point between the two lobes   The geometric centre of the gland, estimated roughly as the midpoint between the centres of the two lobes, did not always coincide with the centre of radiation calculated from the "inverse square" plot   This small discrepancy may be due partly to uneven distribution of the radioactive material, and to the difficulty of judging the position of the centre of gravity   It should be noted, however, that in the estimation of the radioactivity of a thyroid, the distance to the counter should be measured from the centre of radiation   Therefore the indirect procedure for measuring the distance between the thyroid and the counter is preferable to direct measurement of the geometric centre, even in the cases where the latter is possible

We have not examined thyroids of pathological size, but experiments with glass models containing radioactive solutions indicate that the method is valid when the separation of the lobe centres varies from 3 0 to 8 8 cm and when the size of each lobe varies up to 250 ml   Within the limits of these dimensions, the inverse square law is obeyed at all distances greater than about 15 cm from the centre of the source   Since glass and soft tissue have approximately the same absorption coefficient for gamma rays, we conclude from these results that the method is valid for the largest goitres which occur in thyrotoxicosis

In plotting the values of $\frac{1}{\sqrt{a}}$ against d, the line of best fit has been calculated in all cases since appreciable errors may arise if the line is drawn by eye   The line used has been the major axis of the ellipse of correlation, which is drawn through the mean position of all observed points   Its gradient, m, is calculated from the deviations between the co ordinates of these points (x, y) and those of the mean position of all points ($\bar{x}$, $\bar{y}$) by the formula

$$m^\circ = \frac{\Sigma \, (y - \bar{y})^2}{\Sigma \, (x - \bar{x})^2}$$

This line, of which the gradient is the geometric mean between those of the two regression lines, is more suitable than either of these regression lines since neither the values of x nor of y are normal in their distribution

The strength of a source can then be estimated simply from the gradient of the line, since it is known that the line, when extrapolated, passes through the centre of radiation of the source For the slope m is equal to the value of $\dfrac{1}{\sqrt{Q}}$ at unit distance from the source Since the counting rate at a standard distance is a measure of the strength of a source, the value of $\dfrac{1}{m^2}$ can be used as such a measure

We have estimated the effect of the surrounding neck tissue on the external counting rate of the thyroid by two methods In one, an artificial source was measured in a known position in the neck of a living subject In the other, the radioactivity of a thyroid injected with radioiodine was measured when in the neck of a cadaver With these experiments, we also checked the validity of the indirect method for finding the position of a source, when the source is inside the body

The amount of back-scatter from a source in the neck of a living subject

Method A solution containing about 40 microcuries of radioactive iodine was placed in one end of a 3 mm bore graduated rubber tube The column of liquid, 2 cm long, was sealed at each end by a small lead ball fitting tightly inside the tube and serving also to mark the position of the source in an X-Ray photograph The radioiodine content was then estimated from the counting rate at the standard distance with the tube suspended in air The subject then swallowed the tube until it was held between the teeth at a point 10 cm from the lower end With the subject sitting behind the lead screen described in a previous paper (1), a counting rate was obtained with the counter in front of the neck Then, with the counter in the same position, counting rates were observed when the tube was swallowed at various depths increasing by intervals of 0 5 cm As the source came more fully " into the view of " the counter, the counting rate increased, then reached a plateau, and then decreased as the source passed below the aperture in the screen, " out of sight of " the counter The tube was then brought back to the position of maximum counting rate and the source was then at about the level of the cricoid cartilage At this depth the whole of the source was " in view of " the counter All subsequent measurements were made with the tube held between the teeth at this position The position and strength of the source were then measured by the method described in the previous section In all cases, the closest position at which counting rates were obtained was 12 cm from the cricoid The whole experiment occupied about 25 minutes, and gave the subject local radiation exposure of between 0 05 and 0 1 roentgens

The actual distance from the source in the rubber tube to the counter at zero position, was measured as the sum of the distance between the counter at zero position and a point on the skin overlying the cricoid cartilage, and the distance from this point to the source This latter distance was measured from lateral X-Rays of the neck with the tube in position and with a small lead disc on the skin over the cricoid This distance on the X-Ray plate

was corrected for magnification   The magnification factor was determined from the ratio of the distance between the shadows of the lead balls, and their actual separation in the tube
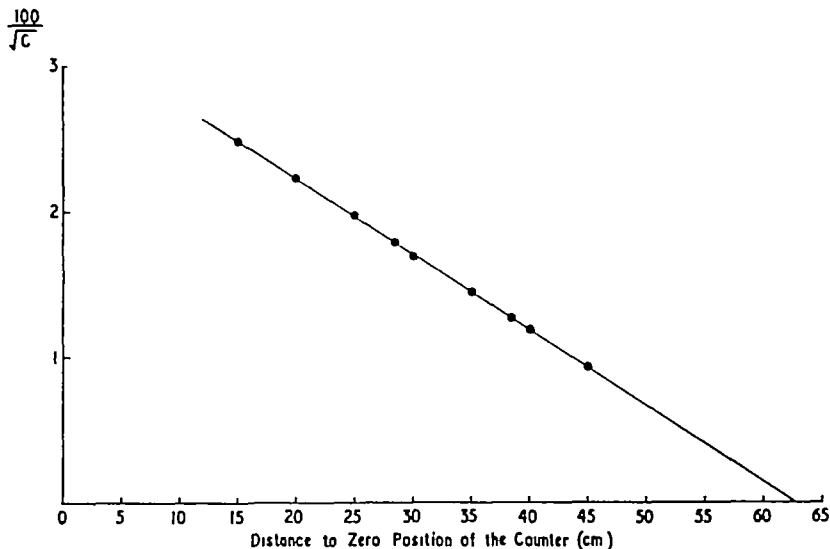

Fig 2   Subject 7   Estimation of the position of a radioactive source in a swallowed tube (C measured in counts per minute)

Results   Fig 2 shows the results obtained in one of 13 experiments with 12 different subjects   In every case, all the points lay close to a straight line, so that the error in calculating D was small and the radioactive solution behaved as a point source in the whole of the range used   Table II shows the results of these experiments   The predicted distance differs by an average of less than 1 cm from the actual distance *   The apparent increase in strength is given in coloumn 5 as a percentage of the true strength   The mean increase is 27 5%, with standard deviation 4 8%   It is clear that the position of the source situated in the midline of the neck can be predicted adequately by this method, and that the apparent increase in strength is approximately constant   The data may therefore be used to predict the true strength from the apparent strength in any given experiment   The best estimate of the true strength in a single experiment would be $\dfrac{100}{127\ 5}$ = 78 5% of the apparent strength

* The small systematic error in estimating the position of the tube may be due, at least in part, to the relationship between neck and lead screen   Since the neck is a few centimetres behind the screen, the counter   sees " rather more neck tissue, and therefore more scattered radiation, when close to the screen than when far from it   The same factor however operates equally in determinations on thyroids so that no error should be introduced when calibration of the thyroid counting rate is based on that derived from experiments with the tube

TABLE II

Subject	Distance between zero position and —		Difference (Dr — Dg) in cm	Apparent* increase %
	geometric centre (Dg) in cm	calculated centre of radiation (Dr) in cm		
1	62 9	62 8	— 0 1	26
2	66 3	64 2	—2 1	24
3	63 3	62 5	—0 8	35
4	64 8	63 9	—0 9	29
5	63 8	62 6	—1 2	26
6	63 2	62 4	—0 8	22
7	64 2	63 0	—1 2	27
8	64 4	64 4	0	32
9	63 7	62 5	—1 2	29
10	65 4	65 9	+0 5	34
11	63 5	63 4	—0 1	33
12ᴀ	62 7	61 5	—1 2	20
12ᴅ	66 3	65 1	—1 2	23 5
			Mean —0 8	Mean 27 5
				S D 4 8*

The amount of back-scatter from a radioactive thyroid in the neck of a cadaver

Method   The thyroid was dissected from the neck of a cadaver and waterproofed by painting with vinyl acetate   A solution of radioiodine was injected diffusely into the gland, the sites of injection being sealed with vinyl acetate   The gland was then suspended in air and its radioactivity found from the counting rate at the standard distance   It was then replaced in the neck of the cadaver in its original position, and the skin incision was sutured   With the cadaver placed in the standard position behind the lead screen, the position and radioactivity of the injected thyroid were found by the procedure described above   At the end of the experiment, the skin incision was opened again without disturbing the position of the thyroid and the distance between the centre of the gland and the zero position of the counter was measured   The gland was then removed and any radioiodine

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* We are indebted to Mr B D Corbett for pointing out that the variability of this increase in different subjects could result from the known errors in the determinations on which the figure is based   We have therefore no evidence that the apparent increase varies significantly in different subjects

which had leaked from the gland into the surrounding tissues was estimated from the counting rate   The experiment was rejected if appreciable leak had occurred   Data were also obtained in a single experiment with an injected thyroid placed in a model neck constructed from human thigh tissues   The thyroid was embedded in the muscle at a distance from the femur approximately equal to the normal distance between the thyroid cartilage and the cervical vertebræ

Results   Table III shows the results from three experiments using cadavers, and from the experiment using a thigh

TABLE III

	Distance between zero position and —		Difference (Dr — Dg) in cm	Apparent increase %
	geometric centre (Dg) in cm	calculated centre of radiation (Dr) in cm		
Body 1	63 0	63 9	+0 9	22
Body 2	64 5	64 8	+0 3	36
Body 3	62 4	61 6	—0 8	19
Thigh	55 3	54 3	—1 0	29
			Mean	26 5

In view of the agreement between the experiments with the source in the rubber tube and with injected thyroids, it appears reasonable to take the mean value from all 17 experiments as an estimate of the apparent increase in the radioiodine content of a thyroid *in vivo*   The actual radioiodine content of a thyroid is therefore assumed to average 79% of the apparent value given by radioactivity measurements on the neck   The true correction in individual experiments should differ from this mean value with a standard deviation of 3% or less

Estimation of the radioiodine content of a thyroid in the living subject

Method   The method described in the first section has been applied to the thyroid gland in subjects who have been given an oral dose of radioiodine   Since the estimation takes about 20 minutes to carry out, it is necessary to wait until the counting rate is stable or changing slowly   This condition is usually reached within a day of the dose, both in thyrotoxic and normal subjects   Counting rates are then measured with the subject sitting behind the screen, and with the counter at different distances from its zero position   We have usually taken readings at six different distances, the nearest at about 17 cm from the centre of radiation and the farthest at about 65 cm

The counting rate at the standard distance from the centre of radiation of the gland is then compared with the counting rate given by the dose at the same distance   The ratio of the two gives the apparent quantity of radioiodine in the thyroid, and the actual quantity is estimated by applying the correction for back-scatter   When the counting rate at one point on the uptake curve has thus been calibrated, all the values taken later than the first few hours may be calibrated, since the counting rate is then proportional to the radioiodine content of the thyroid

In most normal subjects the radioiodine in the body tissues remains high enough for the first two or three hours to influence appreciably the external counting rate at the neck   The counting rate in this early period is therefore not proportional to the amount of radioiodine in the thyroid and the calibration figure obtained at a later period cannot be accurately applied The counting rate observed when the counter is held close to the thigh, however, indicates roughly the quantity of radioiodine in the thigh tissues   In a completely thyroidectomised subject we found the counting rate at the thigh to be about twice that obtained from the neck In normal subjects therefore a rough estimate of the contribution by general neck tissues to the counting rate opposite the thyroid in the early phase can be made from the thigh count   Within two or three hours in normals and earlier in thyrotoxic subjects, the counting rate at the thigh has fallen to a low value relative to that at the neck which may then be assumed to measure mainly the radioiodine in the thyroid
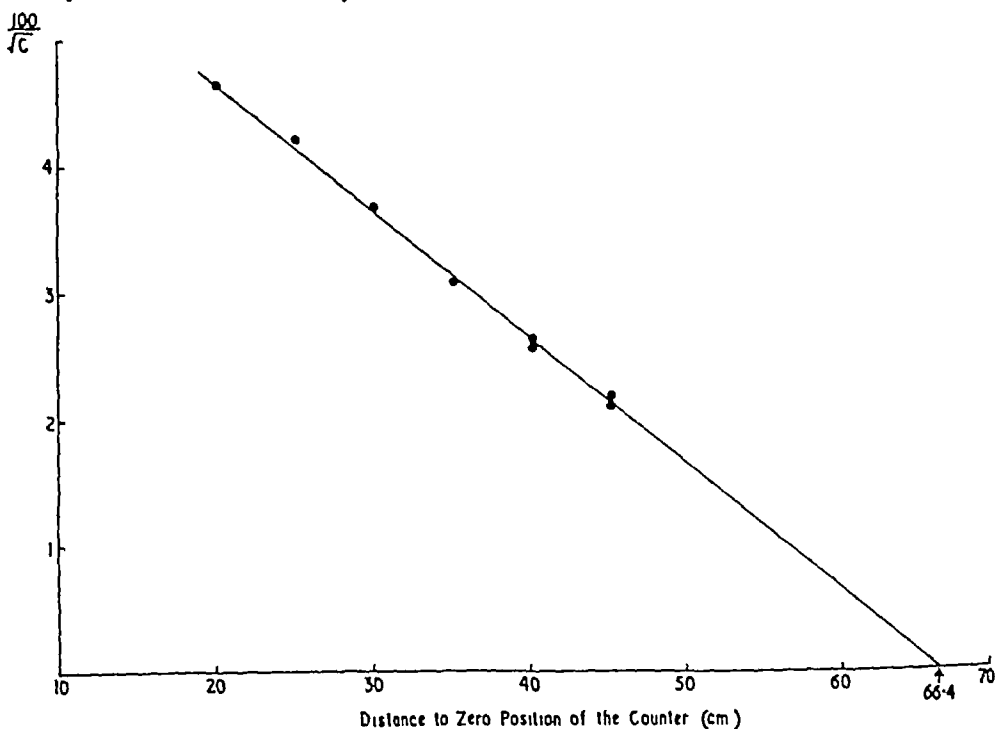

Fig 3   Estimation of the position of the thyroid in a living subject, one day after oral administration of radioiodine

Results   Using these precautions, we have obtained satisfactory results with normal and thyrotoxic subjects with or without goitre   The predicted centre of radiation has been in good agreement with the centre of the thyroid judged clinically and the largest goitres have behaved as point sources over

the range used for estimating their radioactivity   Fig 3 shows the results
obtained with a case of non-toxic goitre of moderate size   The calculated
centre of radiation was 66 4 cm from the counter at zero position, or 3 5 cm
behind the cricoid cartilage   The apparent strength was 34 5 microcuries,
and the true strength was therefore taken as 27 4 microcuries which was
equivalent to 64% of the total dose given on the previous day

Discussion

We have shown that the radioiodine content of a thyroid in air can be
determined by a method in which the gland is treated as a point source
The position of a radioactive thyroid *in vivo* may also be found by this method,
but the apparent radioiodine content *in vivo* must be corrected for back-
scatter   The amount of back-scatter has been estimated by comparing the
true and apparent radioactivity of two artificial sources in the neck   Since
there is good agreement between the values given by sources of such widely
differing shape, it appears justifiable to take the mean value in all experiments
as a measure of back-scatter from a thyroid *in vivo*   However, with estima-
tion of radioiodine in the thyroids of living subjects there may be additional
sources of error, such as differences in the distribution of radioiodine within
the gland   We have therefore obtained a rough check on the method
described here, by calibrating the external counting rate in the same subject
by this and another more indirect method

In four normal subjects, data were available on which the comparison
could be made   The radioiodine content of the gland was measured by the
"inverse square " procedure, with correction for back-scatter   The propor-
tion of the dose in the thyroid was also estimated by difference from the
amount excreted in the urine and from that present at the same time in the
extra-thyroid tissues of the body   The latter was calculated from the plasma
radioiodide concentration assuming an iodide diffusion space of 20 litres
This figure is an approximate average value for normal subjects based on
considerations discussed elsewhere (1)

Table IV shows the values given by the two methods

TABLE IV

Subject	Amount in thyroid estimated by inverse square ' method % dose	Amount not accounted for in urine and iodide space % dose	Difference % dose
1	19 4	22 0	2 6
2	53 5	47 6	5 9
3	64 0	59 4	4 6
4	30 4	31 0	0 6

This comparison is inaccurate because the iodide space was not known exactly, and because a small part of the plasma radioiodine was probably in the form of thyroxine for which the diffusion space appears to be less than for iodide   The agreement between the two methods, however, shows that direct measurements of thyroid radioiodine content can be made with reasonable accuracy by the procedure described

Summary

1   A method is described whereby the radioiodine content of a human thyroid can be determined in absolute units from the counting rate given by a Geiger counter opposite the neck

2   The position of the thyroid centre is calculated from counting rates obtained with the counter at different distances from the neck

3   The apparent increase in radioactivity due to back-scattering of radiation from neck tissues was estimated by comparing the counts from a source when in air and in the neck   In 17 experiments the counting rate observed at a given distance from the source was increased by an average of 27% when the source was within the neck

4   The method gives an approximate but direct estimate of the fraction of a dose of radioiodine which is contained in the thyroid gland

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QUANTITATIVE OBSERVATIONS ON VASCULAR REACTIONS IN HUMAN DIGITS IN RESPONSE TO LOCAL COOLING *

By H. H. WOLFF and E. E. POCHIN

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IN three papers (1, 2, 3) Lewis described certain vascular reactions which occur in human fingers during and after immersion in water at temperatures between 0° and 18°C. After a finger was removed from a cold bath, its skin temperature was found to rise by several degrees above that of a control finger, usually reaching a maximum within 20 minutes of the end of immersion, and then gradually falling to the temperature of the control finger during the next half to one hour. We have studied quantitatively the course of this " after-reaction " following cold to establish how its size depends on the duration and temperature of previous cooling. The reaction is affected not only by these factors but also by the amount of tissue immersed, its temperature immediately following the period of immersion and by the subject's general vasomotor tone. While the local factors could be kept constant in different experiments, the vasomotor tone could only be controlled within wide limits. Accurate comparisons can therefore only be made between the behaviour of opposite hands of the same subject during a particular test. By working at about equal room temperatures it has, however, proved possible to obtain results on different days and in different subjects which can legitimately be compared with each other.

Method

The after-reactions observed in the fingers of normal subjects at room temperatures of about 20°C have usually been sufficient to warm the fingers towards, but not to, body temperature at the height of the reaction. Skin temperature has therefore been a practicable index of blood flow to the finger and has been recorded by means of thermojunctions as described by Lewis (2). The subjects were young, healthy males with no history or evidence of circulatory disturbance or of chilblains on their hands. The third, fourth and fifth fingers of each hand were cooled by immersing them as far as their proximal interphalangeal joints in containers filled with water at the required temperatures, or with crushed ice and water when working

at 0°C   Records were made of the skin temperatures of the immersed third fingers, of the unimmersed thumbs as controls, and of the bath temperatures   When the influence of cooling temperature on after-reaction size was studied, two containers at different temperatures were used, one for the three fingers of each hand   The fingers of the two hands were cooled simultaneously for the same duration of 44 minutes   To study the effects of the duration of cooling, the same three fingers of the two hands were cooled in one bath at 8°C , those of one hand being immersed before those of the other, so that cooling came to an end on both sides simultaneously
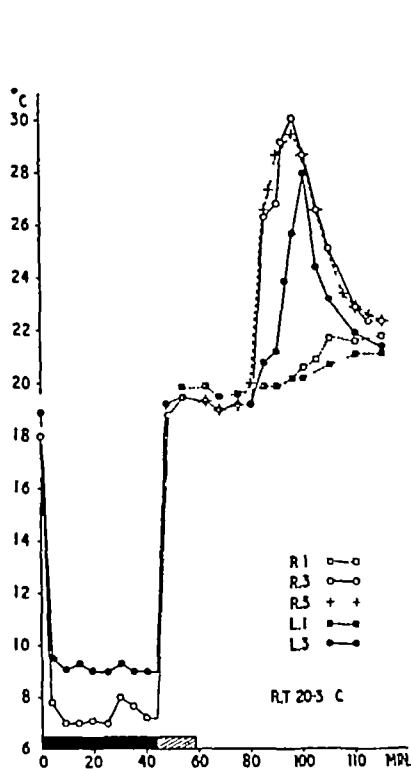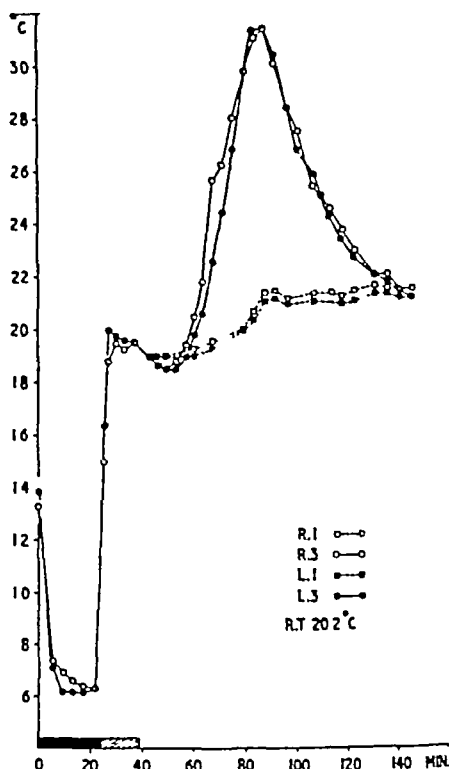

Fig 1   Comparison of after reactions in fingers cooled at 7°C and 9°C   Experiment 2 From 0 to 44 mins , immersion of fingers R3, 4, 5 at 7°C and fingers L3, 4, 5 at 9°C From 44 to 59 mins , both hands immersed to wrist in bath at 19 4°C   Skin temperatures of digits R1, R3, R5, L1 and L3 then recorded for further 70 minutes

Fig 2   Effect of arterial occlusion during cooling on the subsequent after reaction   Fingers R3, 4, 5 and L3, 4, 5 cooled in bath at 6°C from 0 to 24 minutes   Circulation to the left hand occluded by cuff on upper arm during these 24 minutes   Both hands immersed to wrist in bath at 19 5°C from 24 to 39 minutes   Skin temperatures of digits L1, L3, L5, R1 and R3 then recorded for further 110 minutes   Temperature course of L5 closely followed that of L3 and has been omitted from the figure

Since the course of many vascular reactions is influenced by the initial temperatures of the reacting parts, both hands were immersed to the wrist in a bath at 20°C for 15 minutes after the end of the cooling period   The

dried hands were then exposed to air and dry thermojunctions reapplied During the subsequent period of recovery from cooling, the thumb temperatures remained constant or rose slowly as the subject rewarmed Such rewarming was only allowed to occur gradually and provided rapid rewarming was avoided, general vasodilatation of the hands did not occur The temperature of recently cooled fingers at first followed that of the thumbs but after a variable interval the after-reaction started as shown by an abrupt rise of temperature occurring in the previously immersed fingers only These then remained warmer than the control thumbs for an hour or more

TABLE I

Influence of cooling temperature on size of subsequent after reactions in the middle finger The 3rd, 4th and 5th fingers were cooled for 44 minutes

No of Expt	Subject	Bath temp in °C	Size of after reaction		Duration of after reaction in mins	Delay in onset of the after reaction from the end of cooling in mins
			Area* in cm²	Maximum temperature difference above control thumb in °C		
1	H H W	L3 4 8	26 1	7 8	65	76
		R3 10 0	9 8	3 4	57	81
2	H H W	L3 9 0	11 9	7 7	50	36
		R3 7 0	24 4	9 8	55	36
3	H H W	L3 4 0	32 0	7 7	80	91
		R3 12 0	7 7	2 8	65	91
4	H H W	L3 2 0	52 9	12 8	100	56
		R3 14 0	8 0	3 0	70	64
7	H H W	L3 8 0	30 0	10 6	71	37
11	H H W	L3 0 0	81 7	11 8	148	44
9	E D R	L3 11 0	5 6	2 5	53	21
		R3 6 0	38 4	6 9	112	5
17	E D R	L3 3 0	50 8	11 8	78	30
		R3 8 0	19 0	7 0	68	33
10	I J E	L3 3 0	82 7	13 9	113	14
		R3 9 0	42 8	11 6	80	22
16	I J E	L3 12 0	0	0	0	—
		R3 6 0	39 6	11 3	87	14
23	I J E	R3 8 0	8 5	4 3	30	90
20	E E P	R3 8 0	44 2	9 7	120	27

* Here and subsequently, an area of 1 cm.² corresponds to a temperature difference of 1°C for 10 min. or equivalent values

(*see* Fig 1) The after-reaction could easily be distinguished from general vasodilatation because, when this was produced in these subjects by heating the legs, the thumb and finger temperatures rose together and about equally

To estimate the size of after-reactions, the skin temperatures of the third finger and the control thumb were plotted against time and the area lying between those two curves was measured over the whole course of the after-reaction until the finger temperature returned to that of the thumb In so far as skin temperature is proportional to bloodflow, the area measures the additional bloodflow due to the after-reaction The area depends both on the temperature rise and on the total duration of the after-reaction, and is more accurately estimated than either of these factors individually

Results

The influence of the cooling temperature on the size of the after-reaction Table I gives the results of observations on four normal subjects whose fingers were cooled for the same duration of 44 minutes but at different temperatures The measurements all refer to the third finger After-reactions were simultaneously recorded in one other finger of the same hand, usually the little finger, but as the temperature curves of the two fingers followed each other closely, measurements are given only for the middle
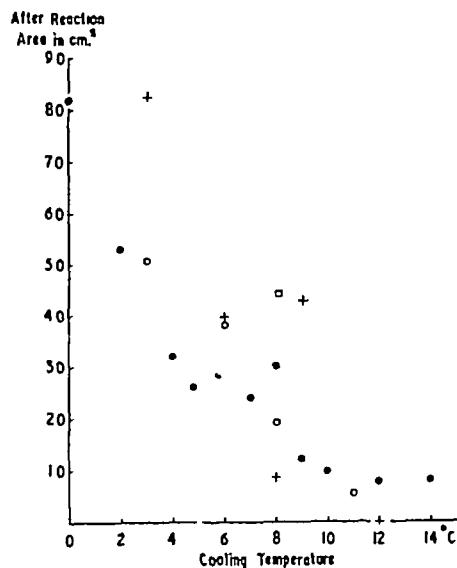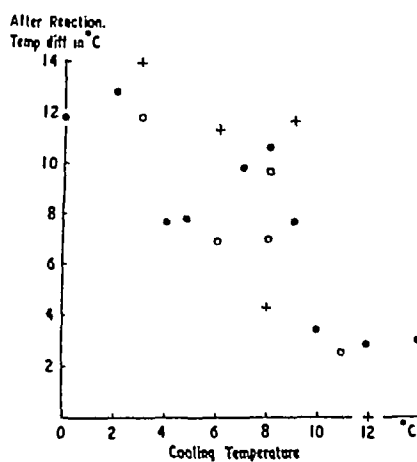

Fig 3

Fig 4

Fig 3 Influence of cooling temperature on subsequent after reaction Size of the reaction expressed as area in cm ² between the temperature curves of the reacting middle finger and control thumb, an area of 1 cm ² corresponding to a 1°C difference for 10 min or equivalent values The 3rd, 4th and 5th fingers were cooled for 44 minutes Different symbols refer in this and subsequent figures to different subjects

Fig 4 Influence of cooling temperature on subsequent after reaction Size of the reaction expressed as the maximum temperature difference in °C between the reacting middle finger and control thumb The 3rd, 4th and 5th fingers were cooled for 44 minutes

finger   Fig 1 is a typical example in which the after-reactions of fingers cooled at 7°C were compared with those of opposite fingers cooled at 9°C When the fingers of two hands were compared, the after-reaction in the fingers cooled at the lower temperature was always the larger (Table I) Results are also given for four experiments in which one hand only was cooled for this duration

Fig 3 shows the relationship between the cooling temperature and the size of the subsequent after-reaction, expressed as the area between the two temperature curves   The size of the after-reaction increases about linearly as the cooling temperature is lowered   Fig 4 demonstrates the similar relationship between cooling temperature and the greatest temperature difference between third finger and thumb reached during the after-reaction It is seen that the size of after-reactions depends on the temperature at which the fingers are cooled, and this relationship is not modified even if the reactions are delayed for as long as one and a half hours from the end of immersion Under the conditions described, the highest cooling temperature which gives rise to after-reactions is approximately 15°C   Six observations were made on one subject (H H W) and two on another (E E P) in which the 3rd, 4th and 5th fingers were always cooled at 6°C for 24 minutes   The results are given in Table II and show a reasonable constancy of the results from day to day and in the two subjects

TABLE II

The size of after reactions in the middle finger after cooling of the 3rd, 4th and 5th fingers at 6°C for 24 minutes

No of experiment	Subject	Size of after reaction.	
		Area in cm²	Maximum temperature difference above control thumb in °C
15	H H W	L3 36 8 R3 40 0	10 2 10 2
19	H H. W	L3 21 1	8 5
21	H H W	L3 32 5 R3 25 0	8 9 6 9
28	H H W	L3 21 4	6 6
29	E E P	L3 30 9 R3 35 5	9 7 9 9

Mean = 30 4   Mean = 8 9

Standard Deviation = 7 2   Standard Deviation = 1 4

TABLE III

Influence of the duration of cooling on the size of the after reaction in the middle finger after cooling of the 3rd, 4th and 5th fingers at 8°C

No of expt	Subject	Duration of cooling in mins	Size of after reaction		Duration of after reaction in mins	Delay in onset of the after reaction from the end of cooling in mins
			Area in cm²	Maximum temperature difference above control thumb in °C		
7	H H W	L3 44 R3 22	30 0 17 0	10 6 8 0	71 53	37 43
8	H H W	L3 11 R3 88	0 98 3	0 12 2	0 150	— 32
17	E D R	R3 44	19 0	7 0	68	33
20	E E P	L3 88 R3 44	80 3 44 2	10 9 9 7	134 120	23 27
23	I J E	L3 16 R3 44	0 8 5	0 4 3	0 30	— 90
18	H H W	L3 120 R3 60	38 4 65 7	10 8 12 5	87 120	23 23
25	H H W	L3 150 R3 75	46 2 77 5	2 3 13 2	83 134	27 25
32	H H W	L3 180 R3 100	24 1 19 7	6 0 4 8	71 71	164 164

The influence of the duration of cooling on the size of the after-reaction Table III gives the results of experiments in which the fingers of the two hands were cooled at the same bath temperature of 8°C but for different durations It will be seen that for durations up to 1½ hours the finger cooled longer always gave larger after-reactions than the finger of the opposite hand cooled for a shorter period This is illustrated in Fig 5 in which the size of the after-reaction, in terms of the area measured, is plotted against durations of cooling for periods up to 1½ hours The size of the after-reaction is about linearly related to the duration of previous cooling This is also true if the size of the after-reaction is expressed in terms of the maximal temperature difference between the reacting finger and control thumb If cooling is continued for over 1½ hours, the subject and his arm often become severely cold, the after-reaction is long delayed and may be slight or transient when it occurs It seems likely that a persistent high general or local vasomotor tone may diminish the after-reaction after such long periods of cooling

Delay in onset of after-reactions When the hands are removed from the bath at 20 °C and placed in air, the fingers at first reach and remain at room temperature During this period before the after-reaction starts, the nailbed of previously cooled digits is seen to be deeper in tint than that of control digits It has been observed that after-reactions are thus delayed while the subject feels generally cold, but develop when he begins to rewarm
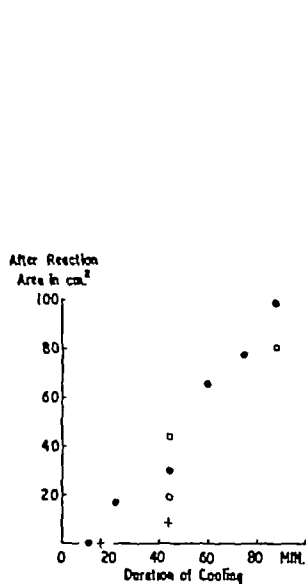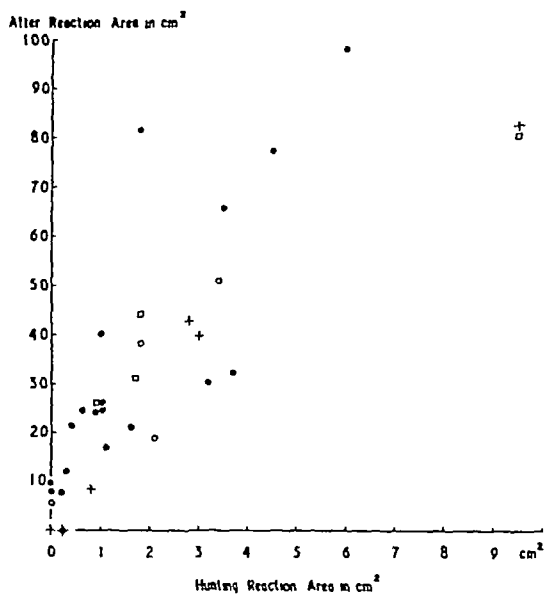

Fig 5

Fig 6

Fig 5 Influence of duration of cooling on subsequent after reaction Size of the reaction expressed as the area in cm² between the temperature curves of the reacting middle finger and control thumb The 3rd, 4th and 5th fingers were cooled at 8°C

Fig 6 Relationship between size of hunting reactions during cooling and size of the subsequent after reaction in the same finger

Size of hunting reactions expressed as the total area in cm² between the temperature course of the immersed finger and the bath temperature Size of the after reaction expressed as the area in cm² between the temperature curves of the reacting finger and control thumb The 3rd, 4th and 5th fingers were cooled at various temperatures and for durations up to 1½ hours Measurements refer to reactions in the middle finger

After a period of delay which has varied from 5 to 164 minutes, the after-reactions in the two hands start abruptly and usually within a few minutes of each other although the hands have previously been cooled unequally Continued local cooling at 20°C may also prevent the after-reaction from developing This was shown by immersing three fingers of both hands at 6°C for 24 minutes and then transferring the hands to a bath at 20°C One hand was kept immersed in this bath for 90 minutes whilst the other was taken out after 15 minutes An after-reaction began in the fingers of the latter after 30 minutes and ran its full course during the next hour, but no after-reaction occurred in the fingers of the opposite hand during the period

of continued immersion at 20°C   It appears therefore that the after-reaction is delayed, not according to the degree of previous cooling, but until the general and local vasomotor tone falls and allows the more proximal blood vessels to re-open   When it occurs, however, its size is, within wide limits, proportional to the degree and duration of previous cooling

The effect of arterial occlusion on the after-reaction   The effect of circulatory occlusion during the cooling period on the size of a subsequent after-reaction has never been adequately established   In a few experiments, we have found that the after-reaction is unaffected by circulatory occlusion during cooling   In Fig 2 are shown the results of an experiment in which the third, fourth and fifth fingers of both hands were cooled simultaneously in a bath at 6°C for 24 minutes   The circulation to the left hand was kept occluded during this period by a pressure cuff inflated at 180 mm of mercury   On removal from the cold bath, the occlusion was discontinued and, after the usual immersion of both hands in a bath at room temperature for 15 minutes, the after-reactions in the two middle fingers were recorded   As shown in Fig 2, the subsequent after-reactions in the third fingers followed each other very closely and did not differ in size   Similar experiments showed that arterial occlusion in the interval between the cooling period and the onset of the after-reaction also fails to influence the size of the reaction

During these occlusion experiments, release of the circulation was as usual followed by a period of reactive hyperæmia   This preceded the after-reaction and was accompanied by only a small and transient rise of skin temperature as compared with the larger and longer rise during the after-reaction   Using the same area measurements for both reactions, the area of an after-reaction following cooling at 6°C for 24 minutes was approximately 20 times as great as that of a reactive hyperæmia in the opposite hand following a circulatory arrest of equal duration at room temperature   It follows that anoxia due to vasoconstriction during cooling cannot contribute appreciably to the size of after-reactions

Size of vasodilator " hunting reactions " during cooling   The course of the recurrent vasodilator " hunting reactions " occurring during cooling and described by Lewis (2), has also been followed during the periods of immersion, and the mean skin temperature rise produced by them has been estimated by dividing the area included between finger and bath temperature curves by the duration of cooling   The mean rise observed decreases progressively as the cooling temperature is increased from 3° to 10°, above which these hunting reactions have been slight or absent under the conditions of immersion that we have used   As both hunting and after-reactions have been shown to increase as the cooling temperature is lowered, it follows that the size of after-reactions will also increase with the size of the preceding hunting reaction in the same finger   This relationship is shown in Fig 6

Discussion

Quantitative studies of the hunting and after-reactions have been made so that they could be compared with similar data from subjects liable to chilblains   In particular, it seemed possible that the after-reaction, which may continue for several hours after suitable cooling even in normal subjects, might be the basis for the vasodilatation and œdema of a developing chilblain in an affected subject   It is clear from the present work that the relevant abnormality in such subjects might be one of several kinds   abnormal tissue cooling under given conditions of exposure , an altered temperature threshold for after-reactions , an abnormal delay of after-reactions by local or general vasomotor tone , an undue duration or degree of after-reactions after a given cooling , or possibly an impaired circulation during cooling from absence or reduction of hunting reactions   The swelling might also depend on the degree of capillary permeability to protein during the after-reaction, or on the rate of lymphatic removal of any interstitial fluid so formed   It was therefore desirable to study the after-reaction quantitatively and to know the degree and duration of cooling at which it reached its maximum size in normal subjects   It may be mentioned that measurements of finger volume have shown no significant changes during or following the after-reaction in normal subjects under any cooling conditions that have been used

It is normally assumed that the after-reaction is caused by chemical substances liberated during cooling as a result of tissue injury   An increase in size of after-reaction with a greater degree or duration of cooling is clearly consistent with this view, if greater cooling causes increased tissue injury   It is, however, difficult to ascribe the after-reaction to release of freely diffusible substances, since the reaction has been shown to occur after a variable period of delay and its size is not influenced by the length of this delay   It would be anticipated that a diffusible substance would slowly escape during this period and that the size of after-reactions would vary inversely with the length of delay

Two other observations suggest that any chemical substances responsible for the after-reaction must be both stable and fixed in the tissues   Firstly, the reaction is often sustained for over two hours, despite the rapid bloodflow through the part during this period   Secondly, it is not reduced in size, even if the bloodflow of the part has been increased by a reactive hyperæmia occurring between the period of cooling and the onset of the after-reaction

It appears further that any substances responsible for the after-reaction are insufficient to cause or maintain dilatation of the more proximal blood vessels, since otherwise the reaction would not be delayed until a general fall in vasomotor tone took place   We have also seen temporary interruptions of after-reactions which could be ascribed to general vasoconstriction from body cooling   Dilatation of the small vessels, as shown by the deepened

tint of the nail bed, is however present immediately after cooling and throughout the delay which precedes the after-reaction   It appears therefore that the after-reaction is not manifested until relaxation of tone in more proximal vessels allows a rapid bloodflow through the already dilated small vessels

SUMMARY

1   A method is described by which the vasodilator after-reactions occurring in recently cooled fingers can be estimated quantitatively

2   The magnitude of these reactions increases as the cooling temperature is lowered

3   The magnitude of these reactions also increases with the duration of cooling up to $1\frac{1}{2}$ hours

4   The after-reactions can be delayed by as much as 2 hours or longer and this delay depends on the subject's general and local vasomotor tone

5   After-reactions are characterised by vasodilatation persisting for as long as 2 to 3 hours in some experiments

6   Circulatory arrest during or after exposure to cold does not alter the size of the subsequent after-reaction

7   Quantitative data on the hunting reactions occurring during cooling are also recorded

8   The possible significance and mechanism of the after-reaction is discussed

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THE IMPAIRMENT OF SENSATION IN BURNS AND ITS CLINICAL APPLICATION AS A TEST OF THE DEPTH OF SKIN LOSS

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THE early diagnosis of the depth of burn damage is becoming increasingly important with the development of immediate grafting of full thickness loss The appearance of a burn sometimes gives a clear indication of the depth but there are many cases which remain doubtful and in which accessory tests would be valuable

It has been recognised for many years that burns of different depths retain different sensory powers Dupuytren (4) in 1832 mentioned changes of sensibility following burning and more recently Cope and others (3) state that sensation is lost in full thickness burns and Colebrook and others (2) found that a series of second degree experimental burns were partially anæsthetic to pin-prick The present study was undertaken to investigate the sensory responses of skin damaged in different degrees by burning and to devise if possible, from these findings, a practical test of burn depth Early experiments showed that alterations in the response to painful stimuli showed greatest promise of providing such a test, pain was therefore examined in greater detail than the other modes of sensation

A detailed investigation of experimentally produced burns was made because observations on patients are not easily controlled and opportunities for repetition are limited by clinical factors The correctness and practicability of tests derived from these experiments were assessed by a detailed study of the burns of six patients

I —EXPERIMENTALLY PRODUCED BURNS

Methods

The burn Burns were induced on both of us with a burner (Fig 2) of the type described by Lewis (5) This consists of a solid brass cylinder weighing 1 kg , having a small cylindrical projection 1 6 cm in diameter Before being used the burner was heated for several minutes in a constant

temperature water bath , it was then rapidly removed from the bath, dried, held in a towel, and the surface of the small projection was lightly pressed onto the skin for the time required   The burns were made either on the extensor surface of the forearm or on the calves

If later there was loss of the superficial skin layers, the burns were treated by simple occlusion with a transparent nylon film of the type described by Bull, Squire and Topley (1)

The depths of the burns were judged from their subsequent clinical progress   For example, a burn of 62°C for 30 sec which epithelialised from below and healed completely in 23 days was taken to be a deep partial thickness lesion, while a burn of 65°C for 30 sec on the same subject which epithelialised from the edges and took 6½ weeks to heal was assumed to be a full-thickness lesion   The former showed no signs of clinical infection , the latter was sterile through most of its course

The stimuli   As standard pain stimulators a series of weighted needles were made (Fig 4)   Small sewing needles were found to be suitable   A knob of solder was made at the eye of the needle and nearer the point a second larger mass of solder was attached, so as to make the weight up to 3 or 6 grammes   Each weighted needle was then mounted in a holder of heavy gauge copper wire bent so as to support the needle loosely between the two masses of solder   The point of the needle could thus be poised over the area to be tested and then lowered so that the full weight was acting upon the skin   A constant stimulus could therefore be given and the responses of the subject compared for different areas and with those of other subjects   The complete needle and holder could be boiled for sterilisation or dry-sterilised in a glass tube with a cotton wool stopper in which it could be kept ready for use

So that stimuli should be regularly spaced inside and outside the burn area a transparent radial grid was prepared   This was made of photographic film from which the emulsion had been removed , when necessary, it was sterilised by boiling   This grid had six radiating lines of holes spaced at 2 mm intervals, and three concentric circles divided it into a central circle corresponding to the burn and two annuli 0 8 cm apart (Fig 3)   The central circle and the two annuli each contained 24 holes   When in use, the grid was centred over the burn and the skin under each of the holes was stimulated once with the weighted needle , consecutive stimuli were given at some distance apart

The stimulator for hot and cold sensation consisted of a copper rod maintained at a constant temperature by a water jacket (Fig 5)   Most of the rod was immersed in a test tube containing either hot water or an ice-water mixture   The free end of the rod projected through the bung of the tube and was rounded for application to the skin   The stimulator was used by moving it slowly over the skin surface

Touch sensation was sometimes reported when the pain and temperature stimulators were applied   In some burns with intact skin, cotton wool and Von Frey's hairs were also used

Technique of observation   During the sensation tests the subject sat without looking at the area being stimulated and with the experimental area supported horizontally   At the beginning of each complete experiment the area to be burned was marked on the skin   A control investigation of the sensation in and around this area was made before burning and another investigation was made immediately after burning   Further observations were made at intervals subsequently until the sensation in the burned area had returned to normal

When a pain stimulator was used the subject reported whether —

(a)   there was no sensation   " 0 "
(b)   there was a sensation of touch only   " 0 " Touch
(c)   there was a sensation of pain similar to that
        experienced from normal skin   " 1 "
(d)   there was a severe sensation of pain similar to
        that experienced from hyperalgesic skin   " 2 "

For convenience these were designated " 0 ", " 0 " Touch, " 1 " and " 2 " as shown   The experimenter recorded the subject's observations on an enlarged diagram of the grid so that the results were recorded in their position relative to the burn

Observations

Observations were made on a series of experimentally produced burns ranging in severity from slight erythema of the skin to full thickness lesions   The sensibility to pain stimulation was investigated in all the burns , sensibility to heat, cold and touch was investigated in some of the burns

(1)   *Hot and cold sensibility*   Hot and cold sensitive spots in the skin are much more sparsely distributed than touch and pain receptors   For this reason an apparent loss of hot and cold sensation in a small burn may not be significant   Furthermore several cold spots were found after 1 and 5 days in a full-thickness skin loss burn   Because of this dispersed punctate distribution of the sensitive spots and the presence of cold sensation in a full-thickness burn it seems unlikely that temperature sensibility can provide a satisfactory test of burn depth

(2)   *Touch sensibility*   Some of the stimuli with a 3 gm   needle on normal skin give rise to a sensation of touch, the remaining stimuli being felt as painful   On stimulating partial thickness burns more of the responses are of touch only and this proportion rises with increasing depth of burning   In addition, some stimuli may give rise to no sensation at all when there is deep partial skin loss   Complete anaesthesia of an experimental burn was hardly ever observed although in full thickness skin loss a large proportion

(70 to 90%) of the stimuli during the first few days after burning may give rise to no sensation and the remainder to a sensation of touch only Most stimuli with a 6 gm needle or with a blunt stimulator in such a case could elicit a perceptible, though reduced, touch sensation

Our observations thus suggest that touch sensibility is only seriously impaired in full thickness burns and even in these there may be sufficient persistent touch sensation to make a clear judgment of depth of burning impracticable

(3) *Spontaneous pain* Immediately after burning there was a pain-free interval lasting up to 25 minutes There then followed a period of spontaneous soreness Neither the time of onset nor the duration of this soreness seemed to have any simple relationship to the severity of the burn For example, five burns of differing severities on one subject gave the following results

Burn	Severity	Onset of spontaneous pain after burning	Duration of spontaneous pain
55°C for 30 sec	Erythema only	7 mins	12 mins
60°C for 30 sec	Erythema only	17 mins	73 mins
60°C for 30 sec	Partial thickness burn	2 mins	118 mins
62°C for 30 sec	Deep partial thickness burn	24 mins	6 mins
65°C for 30 sec	Full thickness burn	Slight pain 30 mins	5 mins
		More severe pain 75 mins	45 mins

The spontaneous pain during this period could be relieved by cooling and recurred on re-warming the burned skin The critical temperature at which the pain reappeared gradually rose as the painful state passed off After pain had ceased to be felt at room temperature it might still return when the burned skin was immersed in a hot bath Trauma or infection of the burn gave rise to a further period of spontaneous soreness

(4) *Pain on stimulation* From the notation adopted each pain response may be characterised The pain sensation of an area, however, can only be completely described by stating the relative proportions of " 0 ", " 1 " and " 2 " responses We found that the skin damaged by burning gave a higher proportion of " 0 " responses than normal skin and the skin around the burn gave some " 2 " responses The degree of analgesia or hyperalgesia is shown by the proportion of "0" or "2" responses observed, since normal skin gives mainly " 1 " (with a few " 0 ") responses

Hyperalgesia occurred in the skin surrounding the burn after all but the least severe burn in this series (55°C for 30 sec) The maximum hyperalgesia occurred within 40 mins and on the same subject its intensity, judged subjectively, was the same after burns of different severity The subsequent duration of the hyperalgesia bore no simple relation to the severity of the burn For example, three burns made on the same subject, one causing only erythema (60°C for 30 sec), one causing a deep partial thickness lesion (62°C for 30 sec) and one causing a full thickness lesion (65°C for 30 sec) each produced surrounding hyperalgesia in which at its maximum 50 to 75% of the stimuli with a 3 gm needle gave a " 2 " response The hyperalgesia around both the erythematous burn and the full thickness burn had decreased within 80 minutes so that only 20 to 30% of the stimuli gave a " 2 " response, whereas the hyperalgesia around the deep partial thickness lesion had only decreased to this extent 4 hours later

In the other subject the hyperalgesia at its maximum was never as great as that described above, only 35 to 55% of the stimuli giving " 2 " responses around similar burns

The extent of the hyperalgesia around one full-thickness burn (65°C for 30 sec) was investigated by using a larger grid At 20 minutes after burning the hyperalgesia extended to 5 cms from the edge of the burn and more than one half of the stimuli with a 3 gm needle gave a " 2 " response , at 80 minutes after burning the hyperalgesia extended to only 1 2 cms from the edge of the burn and only one quarter of the stimuli gave " 2 " responses The edge of the hyperalgesia was fairly sharply de-limited and there were only scattered " 2 " responses beyond the distances stated

Stimulation of the burns themselves showed an increasing degree of hypoalgesia with increasing severity of burning The results obtained during the first day after burning in burns of different severity are shown in the diagram, Fig 1 The responses obtained on the edges of the burns are not included in these results

Three burns (one of 55°C for 30 sec and two of 60°C for 30 sec) causing only erythema showed at the most a transitory loss of pain response to some stimuli This decreased sensitivity to pain lasted for less than an hour after burning and afterwards pain sensation was normal The burn A, in the diagram, was the most severe burn of this type and caused the superficial layers of the skin to peel slightly on the sixteenth day after burning

Two burns causing deep partial thickness lesions (burn D, 60°C for 30 sec and burn E, 62°C for 30 sec) showed a gradually increasing hypoalgesia so that between 2 and 24 hours after burning 40 to 80% of the stimuli gave rise to no pain sensation This proportion decreased to 28% after five days in burn E

Two burns (burn F, 62°C for 30 sec, and burn G, 65°C for 30 sec) causing full thickness lesions showed almost complete analgesia in which more than 80% of stimuli gave rise to no painful response from 1½ hours after burning, until 27 days in burn G and until more than 11 days in burn F Burn F became clinically infected after the 14th day, and thereafter a proportion of " 1 " and " 2 " responses were found
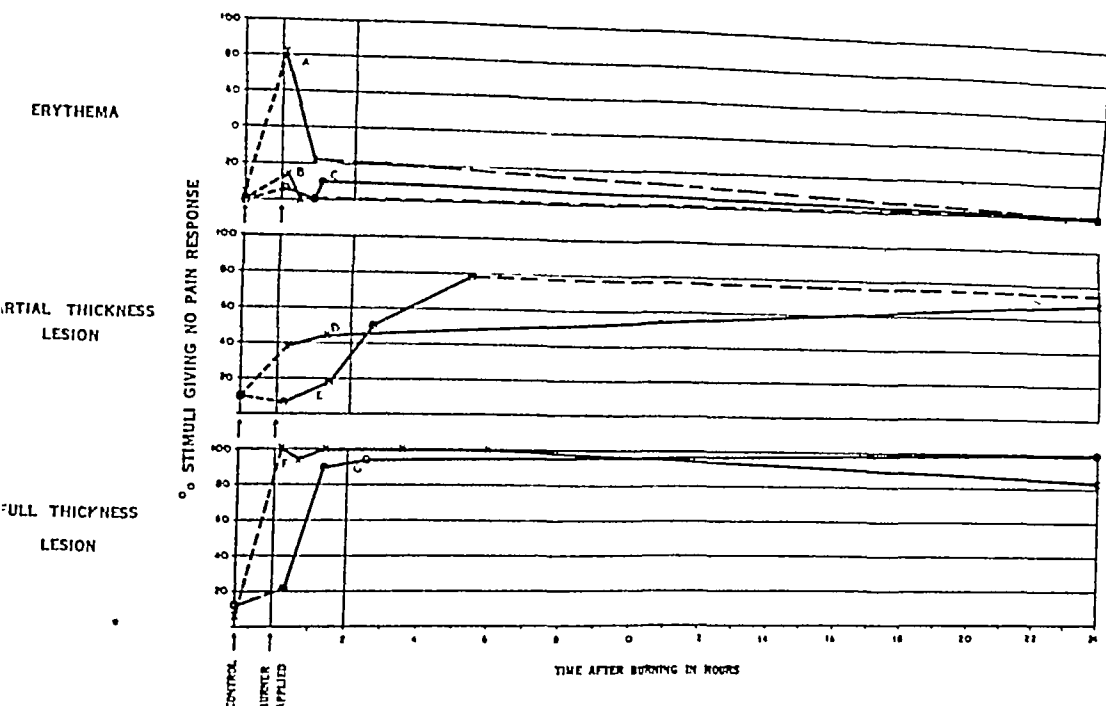

Fig 1   The hypoalgesia of experimental burns of different severity shown as the proportion of painless responses to the weighted needle

II —ABSENCE OF PAIN RESPONSE ON STIMULATION AS A GUIDE TO BURN

DEPTH

The observations described have shown that in a burn many stimuli with a weighted needle produce no pain, these may be felt as touch or not felt at all   The relative proportion of painful to non-painful responses depends on the severity of the burn   This criterion may be made the basis of a test of burn depth

The description to be given of the sensation expected in different types of burn assumes that the tests are made between 2 hours and 2 days after burning   Before this time our results show great variation in the responses, after this time pain sensation may return almost to normal in partial thickness burns although there is little if any pain sensation in a full thickness

burn until much later   Clinically the test may be made with a standard weighted needle as described or failing this, the same general results can be obtained using a sterilised hypodermic needle applied with similar pressure When the stimulus is felt, it is important to find out whether the sensation is that of " touch " or " pain "

Burns causing only very slight skin damage such as those produced by ultra-violet radiation may show a temporary hyperalgesia of the burn area   A more usual finding in slight thermal burns, presenting clinically an erythema with or without late superficial blistering, is that the responses to pricking stimuli are similar to those of normal skin with perhaps a few (0 to 2 out of 10) of the stimuli felt as touch rather than pain   With increasing degrees of partial thickness damage this proportion of painless responses increases   Burns with deep partial thickness lesions give up to 8 out of 10 non-painful responses but few stimuli are completely undetected   Burns with full thickness lesions give a very high proportion (8 or more out of 10) of non-painful responses and of these a high proportion (7 out of 10) in turn give no sensation at all if a light stimulus is used   If a heavier stimulus (6 gm) is used the high proportion of non-painful responses remains unaltered, but the non-painful pricks may be felt as touch   Thus the absence of pain is the more certain criterion of burn depth but with practice the absence of touch sensation to light stimuli may be an additional guide

The value of such a test might be limited by two factors   Firstly some of the body regions might be insensitive to the pain stimulus used   Secondly, the hypoalgesia found after burning might be caused by simple physical factors of insulation such as by œdema and not by the degree of damage to the tissues

The whole body surface except for the palms of the hands and soles of the feet was found to be sufficiently responsive to make the test feasible This is demonstrated in Table I which shows the results of 50 observations with a 3 gm needle on each of 18 body surface regions of two subjects

The formation of visible œdema has not been found to cause measurable hypoalgesia   Thus hypoalgesia was established before there was any evidence of œdema formation in two partial thickness and one full thickness burn , in the two former the proportion of analgesic responses remained constant after œdema formation had occurred   As further evidence, no marked hypoalgesia was observed in a partial thickness and a full thickness burn at a time when there was definite œdema, although hypoalgesia developed later

In three burns a plaque of burned skin or slough which formed several days after burning made tests of sensation unreliable until the tissues were again exposed

TABLE I

Results of sensation tests on different areas of normal skin expressed as percentage of stimuli reported painless (50 tests with 3 gm needle on each area of each of two subjects)

	Flexor surface	Extensor surface
	Percentage painless	Percentage painless
Upper limb		
Arm	10	9
Forearm	3	11
Hand and fingers	83	18
Lower limb		
Thigh	11	20
Leg	18	32
Foot and toes	100	24
Face and neck	6	4
Trunk		
Thorax	2	10
Abdomen	10	4
Buttocks		23

III —OBSERVATIONS UPON CLINICAL CASES

Six clinical cases of moderately severe burning were examined in detail on admission and evidence of the true depth of the lesions was obtained from the clinical progress of healing and in some cases by histological biopsy Patients were selected whose burns were not very extensive and who could co-operate intelligently with the tests   They were otherwise a mixed group and comprised one case of electric burns of the hand, one bitumen burn and one flame burn of the forearm, an alkali burn of the back, a metal burn of leg and a steam scald of the buttock   Different areas of these burns gave every degree of damage from erythema to charring

Each case was thoroughly examined within a few hours of the occurrence of the burn   The detailed appearances of the areas studied were noted and drawings and photographs made   The sensation tests were performed with a sterilised weighted needle   Both 3 gm and 6 gm needles were used and the findings corresponded, although the heavier needle gave in general a clearer result   The radial grid was not used, the tests being made at random over the areas studied   The summarised results are shown in Table II

TABLE II

Summary of findings upon clinical cases

Personal details	Area studied	Appearance on admission	Sensation test result (within 1 to 5 hours of injury)	Biopsy result	Clinical course	Conclusion
Case I M 30 yrs Electric burn	Rt index finger	White dermis visible over radial and dorsal surfaces of prox and mid phalanges	White area—anaesthetic to both pain and touch	Full thickness lesion	White area excised and grafted Healed by 20th day	Full thickness lesions of anaesthetic area confirmed by biopsy
	Rt mid finger	White streak along dorsum No blistering	Proximal phalanx and white line — hypoalgesic Remainder of finger —normal sensation		Blistering over prox phalanx by 6th day All healed by 17th day	Partial thickness lesion of hypoalgesic area confirmed by clinical course
	Rt ring finger	Blistering over dorsum of prox phalanx and oedema of ulnar border of mid phalanx	Blister and oedematous area hypo algesic		Open blister of all burnt areas by 6th day showing white floor Retesting showed this hypoalgesic still Prox'l part healed by 17th day All healed by 20th day	Partial thickness lesion of hypoalgesic area confirmed by clinical course
	Rt little finger	White streak along dorsum of prox'l phalanx No blistering	Proximal phalanx hypoalgesic Elsewhere normal		Blister over prox'l phalanx by 6th day Healed by 17th day	Partial thickness lesion of hypoalgesic area confirmed by clinical course
	Dorsum of hand	Knuckles pale with red limiting line proximal to knuckles	Knuckles hypo algesic Area prox'l mal to red line hyperalgesic		Blistering over knuckles by 6th day Healed by 21st day	Partial thickness lesion of hypoalgesic area confirmed by clinical course

TABLE II—continued

Summary of findings upon clinical cases

Personal details	Area studied	Appearance on admission	Sensation test result (within 3 to 5 hours of injury)	Biopsy result	Clinical course	Conclusion
Case II M 32 yrs Splashed by caustic soda solution	Back Central area	Grey floor of open blister	Marked hypoalgesia	Deep partial skin loss	Healed by 25th day	Deep partial thickness lesion of hypoalgesic area confirmed by clinical course
	Back Periphery	Pink floor of large open blister	Moderate hypo algesia		Healed by 15th day	Partial thickness lesion of hypoalgesic area confirmed by clinical course
Case III M 39 yrs Burnt by hot bitumen	Flexor surface of rt forearm	Large broken blister with pinkish white floor	Analgesia	Full thickness lesion	Evident full thick ness loss at 15th day Slough excised and grafted	Full thickness lesion of analgesic area confirmed by biopsy and clinical course
	Flexor surface of rt forearm (distal)	Open blister with pink floor	Moderate hypo algesia		Healed by 14th day	Partial thickness lesion of hypoalgesic area confirmed by clinical course
	Palm of rt hand	Erythema only	Moderate hypo-algesia		Subsequently blistered and healed by 11th day	Partial thickness lesion of hypoalgesic area confirmed by clinical course

Case	Site	Appearance	Sensation	Clinical type	Course / treatment	Conclusion
Case IV M 30 yrs Rt buttock burnt during scalds	Rt buttock central area	Broken blister with whitish pink floor	Marked hypoalgesia	Full thickness lesion	Evident full thickness loss at 16th day. Excised and grafted	Full thickness lesion established by biopsy and clinical course (sensation test ambiguous See text)
	Rt buttock periphery of burn	Broken blister pink floor	Slight hypoalgesia	Superficial partial thickness lesion	Healed by 7th day	Superficial partial lesion confirmed by biopsy and clinical course
Case V M 40 yrs Splashed with molten metal	Lt leg	White area with charring in centre	Analgesia	Full thickness loss	Immediate excision and grafting	Full thickness lesion of analgesic area confirmed by biopsy
Case VI M 25 yrs Acetylene gas pipe exploded causing burns of forearm	Rt forearm central area	Epidermis stripped off leaving white floor	Anaesthetic to both pain and touch	Full thickness loss	Some hours later evident full thickness lesion Immediate excision and grafting	Full thickness lesion of anaesthetic area confirmed by early changes in appearance and by biopsy
	Rt forearm	Pink floor	Moderate hypo algesia	Partial thickness loss	Healed by 13th day	Partial thickness lesion of hypoalgesic area confirmed by biopsy and clinical course

On the whole the expected close relationship between sensation and depth of burn was confirmed　The findings on superficial and partial thickness lesions paralleled the studies on experimental burns, the burnt area being hypoalgesic and the surrounding skin hyperalgesic　The precise depth of burning was confirmed by biopsy in three cases of partial thickness loss, and the subsequent rate of regeneration was followed in all the cases and provided a further check upon the correctness of the diagnosis

Full thickness burning was present and confirmed histologically in five of the cases studied　In four of these the areas concerned were analgesic to the weighted needle　The remaining one (Case IV) gave an occasional positive response to the test　This could only be elicited after a considerable questioning and it was not clear whether these stimuli were felt as touch or pain　The clinical impression at the time was that some pain sensibility had persisted and that the area might be a deep partial skin loss rather than full thickness　The portion taken for biopsy showed an indubitable full thickness lesion and this was confirmed by a repeated biopsy and the subsequent clinical course of the burn　Apart from this ambiguous case the full thickness areas all showed analgesia when tested by the weighted needle

DISCUSSION

Recent work on the anatomy of the skin (6, 7) has demonstrated the presence of a nerve net in the epidermis which is believed to subserve pain sensation　It is reasonable to suppose that the degree of injury to this nerve net will run parallel with that of the surrounding epithelial cells, and therefore that the degree of hypoalgesia will be a useful measure of the depth of skin burning　The present studies on experimental and clinical human burns suggest that this is so　The finding of hypoalgesia with partial skin loss is consistent with a partial damage to the superficial parts of the nerve net and the analgesia of full thickness loss may likewise correspond to complete death or damage of the nerve supply

There are limitations to any test which depends upon subjective responses of the patient and under the stress of a recent injury these responses may be misleading　It is possible that the ambiguous result obtained in Case IV of the clinical series was due to this subjective error　On the other hand the test is quite simple and good co-operation is usually obtained both with children and adults

SUMMARY

1　A standard method for the study of pain sensation in normal skin and experimental burns is described　The method has been simplified for use upon burned patients

2　Results with this test applied to experimental burns, normal skin and clinical burns are presented

3   These results are correlated with the depth of burning estimated by the subsequent clinical course and, in some cases, by biopsy

4   In general partial thickness burns, both experimental and clinical, showed moderately diminished pain sensibility   In full thickness burns on the other hand, the pain sensibility was greatly reduced or completely absent

5   The clinical test is simple and is considered to give sufficiently consistent results to provide a valuable addition to the methods of diagnosis of the depth of skin damage in burned patients

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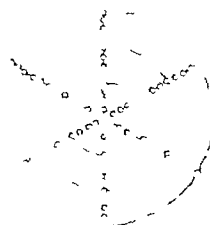(7) WEDDELL   Brit med Bull 1945, 3 167

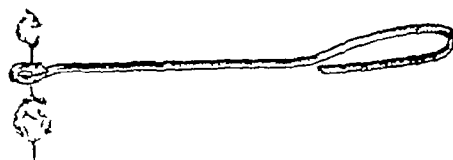Fig 2 The Burner (x 3/5)

Fig 3 The Celluloid grid (x 3/5)
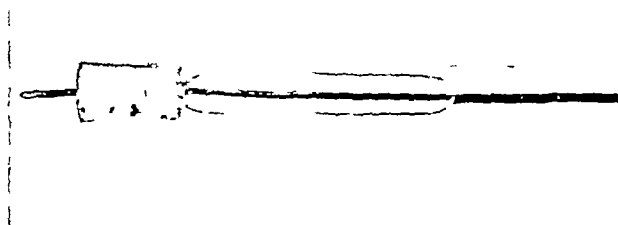

Fig 4 The weighted needle (x 3/5)

Fig 5 The tester for temperature sensation. (x 1/2)

HEPARIN TOLERANCE IN RHEUMATIC FEVER

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Introduction

THE intercellular connective tissue of the human body is made up of two
components, collagen, and interfibrillar ground substance   These two
components can be easily distinguished under the microscope by differential
staining   The interfibrillar ground substance contains polysaccharides, and,
particularly, sulphated polysaccharides such as the mucoitin sulphuric
acids

Histological study of biopsy and post-mortem material obtained from
patients with active rheumatic fever has suggested that the initial injury,
as exemplified by the acute fibrinoid necrosis of the rheumatic nodule,
involved this intercellular connective tissue   It therefore seemed possible
that the hypothetical toxic agent in active rheumatic fever might possess
anti-polysaccharide qualities   Accordingly, the effect of administering a
similar polysaccharide to patients with this disease has been investigated

The only polysaccharide of animal origin readily available in adequate
quantity and purity is heparin   Heparin is a complex, highly polymerized
polysaccharide and contains a variable amount of sulphur, from 12 45 to
13 8% (5)   It is, in fact, a mucoitin polysulphuric acid   Moreover, heparin
has two further advantages   It occurs naturally in the human body, and
by virtue of its marked anti-coagulant power, its presence and physiological
activity can be readily estimated   For these reasons, the action of heparin
was compared in normal individuals and in patients suffering from active
rheumatic fever   A heparin tolerance test was used for this purpose

Material

The material studied was divided into three groups   The rheumatic
fever group consisted of patients being treated in the Special Unit for
Juvenile Rheumatism at Taplow   The majority of these patients were

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children, but some adults were also studied   There were, in addition, two control groups , the first of these consisted of normal individuals, mainly members of the medical and nursing staff of the hospital , the other was a group of patients with active tuberculosis   The material studied is summarised in Table I

TABLE I

Classification of material

A	Rheumatic fever	27 cases
B	Normal subjects	16 cases
C	Active tuberculosis	26 cases

Methods

A heparin tolerance test was first used by De Takats (3)   He gave a standard dose of 10 mg of heparin and estimated the clotting time at regular intervals by the capillary tube method   All his subjects were hospital patients and as a result of this work he divided them into normal, hypo-, and hyper-reactors   Hagedorn and Barker (4) repeated this work, using a larger dose of heparin—25 mg —and employing the Lee and White (6) method for estimating clotting time   They confirmed De Takats' observations

In this present work the heparin tolerance test has again been modified The response in different subjects has been rendered more comparable by varying the dose of heparin according to the body weight of the individual It was felt that this was particularly necessary in view of the wide variations in body weight, especially in children, the group most frequently affected in acute rheumatism   All clotting times were measured by Dale and Laidlaw's technique (2)   This was adopted for the following reasons   An excellent end-point is obtained by this method   Repeated venipunctures are unnecessary, which is a great advantage when dealing with children The results obtained by this technique are very consistent , they are comparable in character and degree, though not, of course, in *magnitude*, with results obtained by other methods   Finally, with this technique the test only takes from one to one and a quarter hours, a considerable saving in time as compared with the technique of Lee and White

Technique of test

A control clotting time is first determined   The patient is then weighed and 1 mg of heparin per kg body weight is injected intravenously   The time at which the injection is given is accurately noted, and clotting times are then estimated at ten-minute intervals for fifty minutes from the time of injection

Results

In normal subjects the maximal prolongation of clotting is usually found at the ten-minute reading, but in some cases the difference between the 10 and 20-minute reading is small   At 50 minutes the clotting time is still considerably prolonged

In subjects with active rheumatic fever the maximal clotting time is very much less, and by 50 minutes the clotting time has returned almost to normal

The results obtained in the normal and in the rheumatic fever groups are summarised in Tables II and III below

TABLE II

Results in normal subjects

Initials	Sex	Age	C C T *	Delay of clotting †					M P T ‡
				10	20	30	40	50	
D.A	M	30	3 5	12 0	5 5	4 0	3 0	2 5	5 4
F.A	F	22	3 0	20 0	7 5	4 5	2 5	3 0	7 5
P B	F	36	3 0	6 0	6 5	4 0	2 5	2 0	4 2
J B	F	28	2 0	11 0	6 0	5 5	5 0	5 0	6 5
K B	M	34	3 0	8 0	7 0	4 0	4 0	3 0	5 2
M C	F	17	3 0	7 5	5 0	5·0	5 5	5 0	5 6
H.F	M	30	2 5	5 0	4 0	3 5	3 5	3 0	3 8
L G	M	39	3 0	18 5	6 5	4 5	3 5	3 5	7 3
R H	F	25	2 5	6 0	5 0	4 5	2 5	3 0	4 2
J H	M	30	2 5	6 5	5 5	4 5	3 0	2 5	4 4
G L	M	25	2·0	6 5	5 5	5 0	4 0	4 0	5 0
S M	F	24	3 5	9 5	7 0	4 5	4 5	3 5	5 8
I.M	F	26	3 0	11 0	8 5	6 0	4 5	4 0	6 8
J.M.	F	25	2 5	10 5	7 0	4 5	4 5	2 5	5 8
J.R	F	17	2 5	6 5	6 5	5 5	4 0	4 5	5 4
F S	M	31	2 5	6 5	7 5	6 5	5 5	4 0	6 0

* C C T = Control clotting time (mins)

† Delay of clotting = Actual clotting time minus control clotting time

‡ M.P T = Mean prolongation time = average of 10 20 30, 40 and 50 min clotting delays

TABLE III

Results in active rheumatic fever

Initials	Sex	Age	ESR*	CCT	Delay of clotting					MPT
					10	20	30	40	50	
G A	F	26	112	3 5	3 0	0 5	1 0	0 5	—	1 0
M A	F	13	67	2 0	3 0	2 5	2 0	0 5	0 5	1 7
E B	M	9	80	2 5	4 5	2 5	2 0	1 5	1 5	2 4
L C	M	14	32	2 5	2 5	2 0	2 5	1 0	1 0	1 8
C.D	F	6	22	2 5	3 5	3 0	2 5	2 0	2 0	2 6
B E	F	18	33	2 5	5 5	2 5	2 5	2 5	2 5	3 1
H F	M	16	32	3 0	2 0	2 0	3 0	2 0	2 0	2 2
W F	M	9	42	2 5	2 5	2 5	2 5	2 5	1 5	2 3
J F	F	13	35	3 5	3 0	2 5	3 0	0 5	1 5	2 1
A G	M	11	20	3 5	7 0	3 0	3 0	2 5	0 5	3 2
B G	F	10	122	2 5	3 0	1 5	1 5	1 5	1 0	1 7
J H	M	13	51	2 5	3 0	2 5	1 5	2 0	1 5	2 1
J H	F	7	27	4 0	2 0	2 0	1 0	1 0	1 0	1 4
A H	M	14	19	3 0	4 0	3 5	4 0	2 0	1 5	3 0
T H	M	14	66	2 5	2 0	3 5	2 5	2 5	2 5	2 6
T J	M	14	50	2 5	3 0	3 0	2 0	1 5	1 5	2 2
J K	M	8	22	2 5	1 5	1 5	2 5	3 5	1 5	2 1
J K	F	8	75	4 0	4 0	2 5	1 5	1 0	1 0	2 0
R.M	M	12	75	2 5	1 5	4 0	3 0	2 5	1 5	2 5
P M	M	7	35	3 0	3 0	2 0	1 0	0 5	1 0	1 5
D P	M	12	75	2 5	3 0	3 5	5 0	1 5	1 5	2 9
C Q	F	29	110	2 5	1 5	1 5	1 0	0 5	0 5	1 0
W S	M	38	20	3 0	3 0	1 5	1 5	1 0	1 0	1 6
R S	M	17	34	3 0	5 0	4 0	2 5	2 0	1 0	2 9
B T	F	6	2†	3 0	1 0	2 5	1 5	2 0	1 5	1 7
A T	F	27	117	3 0	3 0	2 5	1 0	1 0	0 5	1 6
A W	M	13	58	3 5	5 5	3 0	1 5	1 5	0 5	2 4

* All sedimentation rates are the first hour reading by Westergren's method

† This patient was in congestive cardiac failure

Average curves derived from these data are plotted in Fig 1
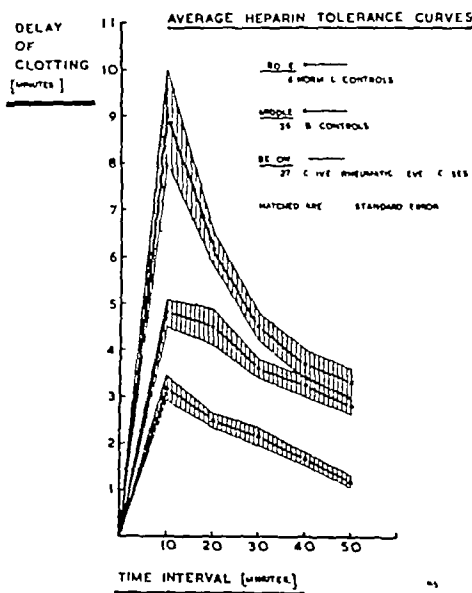
Fig 1   Average heparin tolerance curves in normals (above), active tuberculosis (middle) and active rheumatic fever (below)   The shaded area indicates range limited by ± standard error of the mean
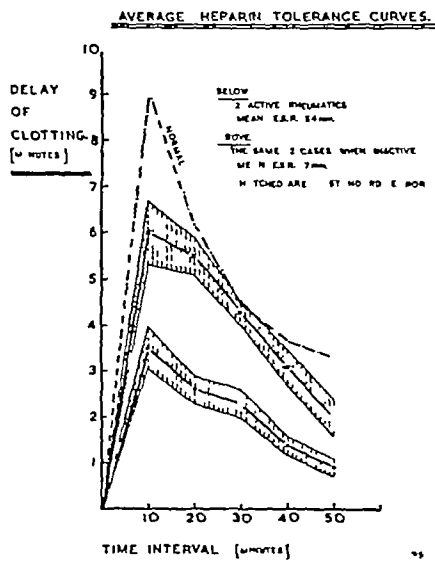

Fig 2   Average heparin tolerance curves during active and inactive rheumatic fever   The interrupted line (above) is the average curve obtained from normal patients.

Curves have been obtained from several patients during rheumatic activity and during the subsequent remissions

Table IV summarises data obtained from twelve patients during activity and inactivity

TABLE IV

Comparison of results during rheumatic activity and inactivity

Initials		Sex	Age	ESR	CCT	Delay of clotting					MPT
						10	20	30	40	50	
G A	(a) (b)	I	26	112 4	3 5 2 5	3 0 12 5	0 5 5 5	1 0 2 5	0 5 0 5	— —	1 0 4 2
L B	(a) (b)	M	9	80 2	2 5 2 5	4 5 7 5	2 5 4 5	2 0 4 0	1 5 3 0	1 5 -2 0	2 4 4 2
J F	(a) (b)	I	13	35 0	3 5 2 5	3 0 5 0	2 5 5 0	3 0 5 0	0 5 3 5	1 5 3 0	2 1 4 3
A G	(a) (b)	M	11	20 5	3 5 3 0	7 0 6 0	3 0 6 0	3 0 3 5	2 5 3 5	0 5 2 5	3 2 4 3
J H	(a) (b)	F	7	27 9	4 0 3 0	2 0 6 0	2 0 4 5	1 0 4 0	1 0 3 5	1 0 2 0	1 4 4 0
A H	(a) (b)	M	14	19 5	3 0 2 5	4 0 7 0	3 5 6 5	4 0 5 0	2 0 4 5	1 5 2 0	3 0 5 0
R M	(a) (b)	M	12	75 5	2 5 3 0	1 5 4 0	4 0 7 0	3 0 5 5	2 5 5 5	1 5 5 0	2 5 5 4
D P	(a) (b)	M	12	75 8	2 5 3 0	3 0 5 5	3 5 9 0	5 0 5 5	1 5 2 0	1 5 1 0	2 9 4 6
C Q	(a) (b)	F	29	110 15	2 5 2 5	1 5 4 5	1 5 3 5	1 0 3 5	0 5 2 5	0 5 1 0	1 0 3 0
W S	(a) (b)	M	38	20 9	3 0 3 0	3 0 3 0	1 5 4 5	1 5 4 5	1 0 3 0	1 0 3 0	1 6 3 6
R S	(a) (b)	M	17	34 4	3 0 3 0	5 0 5 0	4 0 4 5	2 5 5 0	2 0 3 0	1 0 2 5	2 9 4 0
A W	(a) (b)	M	13	58 5	3 5 3 0	5 5 6 0	3 0 5 5	1 5 5 0	1 5 3 0	0 5 1 0	2 4 4 1

The first readings (a) were obtained during rheumatic activity
The second readings (b) were obtained during inactivity of rheumatic process

Fig 2 shows the average curves derived from results tabulated in Table IV It will be seen that with subsidence of rheumatic activity, the tolerance to heparin is diminished Thus there is a reversion towards normal

Active rheumatic fever is almost invariably associated with an elevated erythrocyte sedimentation rate It was important, therefore, to ascertain the heparin response in individuals in whom the sedimentation rate was

raised from causes other than rheumatic fever   For this purpose a number of patients suffering from active pulmonary tuberculosis were tested Results obtained in this series are summarised in Table V

TABLE V
Results in active tuberculosis

Initials	Sex	Age	E S R	C C T	Delay of clotting					M.P T
					10	20	30	40	50	
I B	F	22	22	2 5	3 5	3 0	3 5	3 5	2 5	3 2
J B	F	24	14	2 5	5 5	6 0	6 0	4 5	4 5	5 3
B.B	F	20	4	2 5	7 0	4 5	5 5	4 5	4 0	5 1
D B	M	20	82	3 0	3 5	4 5	3 5	3 5	2 0	3 4
D B	M	20	65	3 0	3 5	3 5	2 5	1 5	2 5	2 7
D C	M	24	33	3 5	3 5	2 5	2 5	3 5	2 5	2 9
D C	M	24	53	2 5	2 5	3 5	3 5	3 5	2 5	3 1
D D	F	39	30	2 5	3 0	5 0	4 0	3 0	2 5	3 5
T.E	M	21	67	3 0	6 0	1 5	2 0	1 0	1 5	2 4
T E	M	21	28	3 0	6 0	120	4 5	3 5	2 0	5 8
D G	F	17	16	2 5	2 5	2 5	2 0	1 5	1 5	2 0
E.H	F	22	14	2 5	5 5	2 5	2 5	2 5	2 0	3 0
L.H	M	20	20	2 5	6 5	6 5	5 0	5 5	3 5	5 4
J.H	M	19	34	3 0	4·0	7 0	4 5	2 5	2 0	4 0
M K	F	36	60	2 5	2 5	4 5	4 0	3 0	3 0	3 4
M K	F	36	60	2 5	4 5	3 5	3 0	2 5	2 5	3 2
J.K	F	24	6	2 0	7 0	6 5	3 0	4 0	4 0	4 9
B J	M	21	72	2 5	3 0	3 5	2 5	2 0	2 5	2 7
C L	F	29	21	2 5	6 5	5 5	4 5	4 0	4 5	5 0
R L	M	23	30	3 0	5 0	6 5	2 5	1 5	3 5	3 8
P M	M	19	45	3 0	4·0	2 5	2 5	2 5	2 5	2 8
D.M	M	23	35	2 5	8 0	3 5	4 5	4 5	2 5	4 6
M.R	M	22	22	2 5	6 5	6 0	6 0	6 5	4 5	5 9
E.S	F	19	10	2 5	4 5	3 0	4 5	4 0	4 0	4 0
D T	F	26	52	2 5	4 0	3 5	2 5	2 5	1 5	2 8
M W	F	37	15	3·0	6 5	4 5	3 0	3 5	2 5	4·0

The average curve from these 26 tuberculous patients is illustrated in Fig 1, together with the corresponding curves from the normal and the rheumatic subjects   It will be seen that in these patients there is some increase in heparin tolerance as compared with the normal subjects, but that the range is far removed from the rheumatic fever group   The difference between the means as shown by these two curves always exceeds four times the standard error of the difference of these means, and thus is statistically significant

There is therefore some correlation between sedimentation rate and heparin tolerance   This may be shown by plotting the mean prolongation times against the sedimentation rates (Fig 3)
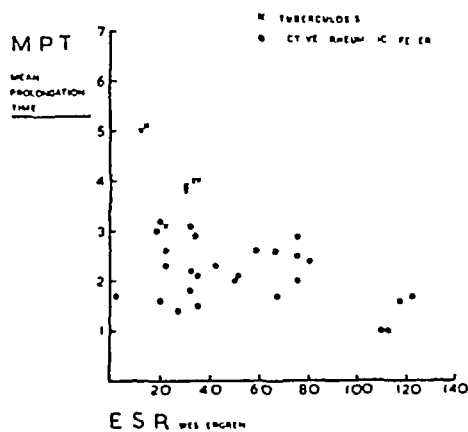
Fig 3   Relationship between mean prolongation time and E S R in tuberculosis and rheumatic fever

This relationship is presented in another way in Fig 4   The patients were divided into five groups according to sedimentation rate as shown in Table VI

TABLE VI

Grouping of rheumatic and tuberculous patients according to E S R

E S R		Group 1 0–20	Group 2 21–40	Group 3 41–60	Group 4 61–80	Group 5 Above 80
T B	No of patients	8	9	5	4	0
	Average M P T	4 2	4 3	3 0	2 8	—
Rheumatic fever (active)	No of patients	4	9	4	6	4
	Average M P T	2 4	2 2	2 2	2 3	1 3

In Fig 4 the average of the mean prolongation times for each group is plotted against the sedimentation rate   It will be seen that the height of the sedimentation rate does not affect the response to heparin in active

rheumatic fever   In tuberculosis, however, the tolerance is increased when the sedimentation rate exceeds 40 mm   although even then there is not such a high degree of tolerance to heparin as there is in rheumatic fever
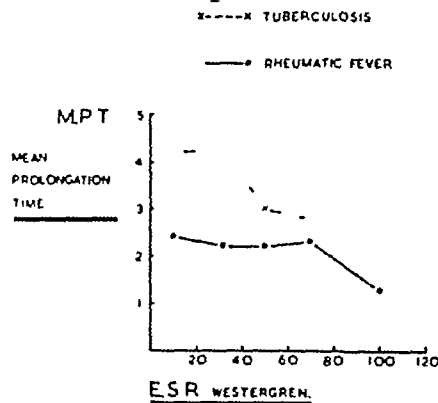
Fig 4   Average prolongation times compared with sedimentation rates in tuberculosis and rheumatic fever   The patients were grouped according to E S R as shown in Table VI

Discussion

These results show that in active rheumatic fever there is a very marked increase in resistance to the anti-coagulant action of heparin   This heparin resistance seems to be very constant and does not depend upon the degree of activity of the rheumatic process as judged by the erythrocyte sedimentation rate   The mechanism of this resistance is at present unknown, but there are several possible explanations   Wilander (8), and Copley and Schnedorf (1) have shown that about 20 to 25% of injected heparin can be recovered from the urine during the first hour   There is little reason to suppose that the renal threshold to this substance is lowered during rheumatic fever, and it is highly unlikely that renal excretion can account for these results   This point is, however, being investigated

It is possible that heparin is taken up from the bloodstream and stored in the tissues   Piper (7) has recently shown that in rabbits, injected heparin may be demonstrated histologically in the reticulo-endothelial system, particularly in the liver and spleen   More probably, however, the heparin is inactivated in the bloodstream, possibly by protein binding   Preliminary experiments have shown that blood from active rheumatic fever patients possesses a resistance to heparin added " in vitro," similar to that which has been demonstrated " in vivo " by the heparin tolerance test

Summary

1   In active rheumatic fever there is a marked increase in resistance to the anti-coagulant activity of heparin as demonstrated by the heparin tolerance test

2   Heparin tolerance bears a direct relationship to rheumatic activity, and with subsidence of this activity the tolerance to heparin reverts towards normal

3   The degree of heparin resistance bears little relationship to the degree of rheumatic activity as judged by the erythrocyte sedimentation rate

4   Further study is necessary of anti-polysaccharide activity in the active rheumatic state

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THE ROLE OF GASTRIC ACIDITY IN THE PATHOGENESIS OF PEPTIC ULCER

By A H JAMES and G W PICKERING *

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PERHAPS the most widely held hypothesis of the pathogenesis of peptic ulcer is that it is due to the action of gastric juice which is peculiar in that it contains abnormal amounts of acid, at least at certain times   Thus peptic ulcer only develops in those parts of the alimentary canal exposed to gastric juice   In the intact subject proved ulcers have never been found in the presence of proved anacidity, and after surgical operation ulcers recur only in those whose gastric juice is acid (20)   It is evident that, in man, acid is a necessary factor in the production of ulcer   That it may be the essential factor is suggested by three types of animal experiment   Mann and his colleagues (10) have shown that operations which deflect the alkaline biliary and pancreatic secretions from the duodenum are followed in dogs by the development of an ulcer or ulcers in the duodenum just where the acid chyme, ejected through the pylorus, may be expected to impinge on the duodenal mucosa   Dragstedt and his colleagues (4) have shown that, while duodenum and jejunum sutured into defects in the gastric wall remain intact when exposed to the ordinary stomach content of dogs, they become digested when exposed to pure gastric juice secreted by isolated pouches, as, indeed, does gastric mucosa itself   Finally, Code and others (5) have shown that gastric and duodenal ulcers may be regularly produced in a variety of laboratory animals by intramuscular injection of histamine in beeswax, which produces a prolonged secretion of highly acid juice

In man the evidence that acid is the abnormal factor is at the present time highly equivocal, and is of two kinds, namely, that derived from test meals and that from aspiration of the stomach contents during the night   Quite apart from technical objections, neither of these methods can give more than a fragmentary picture of gastric acidity   In this paper we describe an attempt to obtain a more complete account of gastric acidity

during the course of a whole day in subjects with gastric and duodenal ulcer and in subjects with no ulcer history   We begin with early experiments on nocturnal aspiration, and our reasons for discontinuing them

NOCTURNAL ASPIRATION OF GASTRIC SECRETION

In the years 1942 and 1943, with the aid of Drs H D Juler and G M Barrett and the Nursing Staff of Harefield Hospital, an attempt was made to find out if the secretory behaviour of the stomach at night was abnormal in patients with peptic ulcer   Seven normal subjects, mainly students and doctors, 12 patients with gastric and 11 with duodenal ulcers were investigated   Four to five hours after the last meal of the day the stomach was emptied through a Ryle's tube, and the tube connected to a receiving bottle and syphon maintaining a negative pressure of about 40 cm of water The collection was continued till morning, when the system was disconnected and the patency of the Ryle's tube confirmed   The aspirate was measured and titrated in the ordinary way, using Topfer's reagent

Fig 1   Shows the rate of secretion of acid during the night, calculated from the volume and the " free acidity " of the total aspirate   In this, as in subsequent diagrams, D U represents patients with duodenal ulcer, G U patients with gastric ulcer, and N subjects without ulcer   Each dot represents a single case, the values being averaged where more than one determination has been done

The results are shown in Fig 1   It will be seen that each group shows a considerable scatter   Of the 12 cases of gastric ulcer two showed very high rates of secretion of HCl   Each of these subjects was investigated twice, and the values shown are the mean of the paired results, which agreed well   Thus, in one subject the rates were 105 and 85 ml N/10 HCl per hour , in the other 52 and 32 ml per hour   The remaining 10 cases of gastric ulcer all showed rates in or below the lower limits of normal   In the 11 cases of duodenal ulcer secretory rates showed a wide scatter, but were in general at a higher level than in normal subjects, and much higher than the majority of patients with gastric ulcer   Here again duplicates showed good agreement, in one case 30 and 38 ml and in another 16 5 and 14 ml N/10 HCl per hour

While it seemed possible that further investigations along these lines might reveal statistically significant differences, we did not pursue them for two reasons. In the first place we felt quite uncertain whether any

differences found represented differences in secretory activity or merely
differences in the efficiency of recovery of the fluids entering the stomach
In the second place the information gained was in a sense not quite the
information we wanted   What we wanted to know was the actual degree
of acidity from hour to hour in the stomach

THE PATTERN OF GASTRIC ACIDITY THROUGH 24 HOURS

In answering the question as to whether an abnormality of intragastric
acidity is or is not the cause of peptic ulcer, we should know the levels of
gastric acidity during the varying circumstances in which the subject finds
himself   We have not yet succeeded in devising a method for doing this
But we considered that if we could record the variations of intragastric
acidity during a whole day under standard conditions, we might obtain a
closer approximation to the answer than has yet been given   We had
intended to do this by means of a small glass electrode which would be
introduced into the stomach and left there, but delays in the delivery of
this led us to devise the following method

24-hour sampling

Samples of gastric juice were removed during 24 hours, and their acidity
determined   All subjects received 4 meals during the day, and drinks of
milk at regular intervals between meals   The meals taken were as follows —

12 noon — Lunch , white fish, potato, carrot puree, fruit puree and custard, cup of tea
2 p m   — Milk.
4 p m   — Tea , crustless bread and butter, tea golden syrup
6 p m   — Supper , white fish, potato, baked custard, bread and butter
8 p.m   — Milk
10 p m — Milk.
6 a.m   — Milk.
8 a m   — Breakfast , porridge, boiled egg crustless bread and butter, cup of tea
10 a.m — Milk

The milk feeds consisted of hot milk or Ovaltine   Care was taken that
nothing was eaten or drunk apart from the prescribed meals   The samples
at the time of meals were ordered to be withdrawn before the meal was
begun, but this was not always carried out

No medicines were given during the tests, alkalis being withheld at
least 24 hours beforehand   The effect of preceding alkali therapy is discussed
later   Smoking was not forbidden

The patients were allowed to get up to toilet, but were otherwise
confined to bed   The 3 normal volunteers were up and about except at
night

The samples were obtained from a Ryle's tube, which was passed,
usually through the nose, at about mid-day   At some time during the
test an X-ray film of the abdomen was taken to ensure that the tip of the

during the course of a whole day in subjects with gastric and duodenal ulcer and in subjects with no ulcer history   We begin with early experiments on nocturnal aspiration, and our reasons for discontinuing them

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No medicines were given during the tests, alkalis being withheld at least 24 hours beforehand The effect of preceding alkali therapy is discussed later Smoking was not forbidden

The patients were allowed to get up to toilet, but were otherwise confined to bed The 3 normal volunteers were up and about except at night

The samples were obtained from a Ryle's tube, which was passed, usually through the nose, at about mid-day At some time during the test an X-ray film of the abdomen was taken to ensure that the tip of the

tube was in the body of the stomach   Some tests were abandoned or excluded because the tube could not be manœuvred a sufficient distance beyond the cardia

Small samples (5—10 ml) were removed every half hour during the day (8 a m —8 p m) and every hour at night   Occasionally it was impossible to get samples , if they were unobtainable for more than two hours consecutively the test was excluded   The small number of tests which had to be left out on this account was not confined to any one group, but was distributed among both ulcer patients and normal subjects

Selection of Cases

The test was carried out on a series of patients with gastric and with duodenal ulcer, and on a group of subjects without ulcer   With the exception of 3 volunteers in the control group, all subjects were being treated as in-patients   No subject, with or without ulcer, who was suffering from an infection or general disorder, such as anæmia, which is known or suspected to be associated with abnormalities of gastric secretion, was included in the series   The test was performed on the patients with ulcer after their spontaneous pain had ceased, but usually before healing of the ulcer was complete

Duodenal ulcer   There were 20 patients in this group   With one exception they had all suffered from epigastric pain which was relieved by food, alkali or vomiting   The remaining case was admitted following a melæna and an unequivocal history of ulcer pain was not obtained   In all but 3 cases, one or more craters were seen radiologically in the duodenal cap, either on the screen or on films or both   In the 3 cases where craters were not seen, there was a deformity of the duodenal cap , in one of these cases the diagnosis of duodenal ulcer was confirmed at operation

Seven patients were subjected to partial gastrectomy , active duodenal ulcers were found in 4, and chronic inflammation and fibrosis of the duodenum in 3   One patient not subjected to gastrectomy had had a perforation " in the pyloro-duodenal region " repaired at another hospital

In 18 cases the stomach was reported as radiologically normal, and in none of the cases subjected to operation was an associated gastric lesion found   In 2 cases small gastric craters were reported by the radiologist in addition to the duodenal craters , these were not seen in either case at subsequent examination, and the duodenal crater was the predominant lesion

Gastric ulcer   There were 23 cases in this group   They had all suffered from epigastric pain relieved by food, alkali or vomiting   In every case X-ray examination revealed a gastric crater which was seen on films, and, in all cases but one, on the screen

Seven patients were subjected to partial gastrectomy, and a benign peptic ulcer was found in each case   In the remaining 16 patients, exclusion of malignancy depended on lasting relief of symptoms by medical treatment, disappearance or great reduction in size of the crater, and, in 4 cases, on the gastroscopic appearance

In no case was there any evidence that the duodenum was also ulcerated The series includes one case of pre-pyloric ulcer, and one case of pre-pyloric ulcer combined with a high lesser curve ulcer, the lesions in both cases being confirmed by operation

Subjects without ulcer   This group was formed of 20 subjects who had never had abdominal pain, vomiting, " indigestion ", or any other symptoms which might have been caused by peptic ulcer   With the exception of 3 volunteers, they were patients in the same wards as those with peptic ulcer, suffering from disorders which were unlikely to affect gastric secretion Febrile or severely ill patients were not chosen   The diagnoses were as follows   3 normal volunteers, carcinoma of sigmoid, hypertension (2), volvulus of sigmoid (after recovery from operation), hysteria, neurosyphilis, collapsed vertebra (2), mitral stenosis and cerebral embolus, muscular injury, myocardial infarction, auricular flutter, varicose eczema, sulphonamide dermatitis, angina of effort, idiopathic epilepsy, carcinoma of lung (symptomless)

Methods of determining acidity

Acidity can be measured by titration or by electrical determination of pH   In this investigation many of the samples contained large amounts of the coloured or insoluble contaminants present in the stomach when ordinary food is being eaten, and the electrometric method was chosen because its accuracy and convenience are not affected by such substances

Fig 2 shows the theoretical relation between pH and hydrogen ion concentration at 25°C in aqueous solutions of hydrochloric acid *   To establish that this relation applies to gastric samples, the acidity of a number of such samples and of some aqueous solutions of hydrochloric acid was determined by both methods ,   the agreement between experiment and theory is reasonably close

Some of the merits and limitations of each method are apparent from the shape of the curve in Fig 2   At high acidities, a small change in pH represents a large change in hydrogen ion concentration, and titration is, therefore, better adapted to the measurement of such acidities

At low acidities the error involved in detecting the end point of a titration becomes serious ,   in any case, because of the presence of buffer substances in gastric juice, titration cannot measure acidities less than thousandth normal

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* Calculated from activity coefficients given by Lewis and Randall, " Thermodynamics and Free Energy of Chemical Substances," New York, 1923

The criticism of pH measurements, that pH is related to hydrogen ion concentration by an activity co-efficient which is itself affected to an unknown degree by any chemical substances which may be present, can be answered in two ways   Firstly, Fig 2 shows that any discrepancy due to such a cause must be very small, the gastric samples behaving in the same way as the aqueous solutions   Secondly, even if the active mass of hydrogen ion, which the electrode measures, differed greatly from hydrogen ion concentration, it would still be the relevant quantity for the purpose of this investigation, which is concerned with hydrogen ions as a possible chemical agent
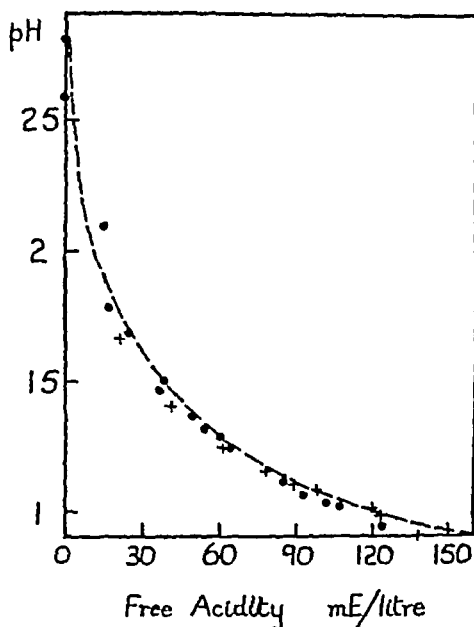
Fig 2   Shows the relation between pH and hydrogen ion concentration   The broken line represents the theoretical relation calculated from the formula

$$pH = \log_{10} \frac{1}{\text{active mass of hydrogen ions}}$$

The experimental points represent the result of determining acidity both by titration and with the glass electrode on samples of gastric contents (dots) and aqueous solutions of hydrochloric acid (crosses)

The sealed glass electrode was used for determination of pH   The instrument was standardized in M/20 sodium hydrogen phthalate (pH 3 97) and M/20 sodium borate (pH 9 20)   A more acid standard was not used as a routine because it was found that such standards were less easily stored, but tests with standard hydrochloric acid or HCl-phthalate buffers showed that pH and potential were linearly related over the whole range of pH's encountered

The electrode was rinsed in distilled water between each reading, an adherent film of mucus might delay a steady reading but did not affect the

final value   Observations in which readings of standard buffer were interpolated among gastric samples showed that the error of an individual determination did not exceed 0 05 pH units

After withdrawal, the samples were kept in corked tubes at room temperature, the determinations being made together after the end of the test In order to find out how much the pH of samples might change while waiting, the determination was made on a number of samples immediately after withdrawal and again after 24 to 72 hours   The results show that a slight change in pH towards the alkaline side occurs with standing, and that this change is smaller in the more acid specimens —

Interval   pH of samples (arranged in order of descending acidity)

Immediate	1 13	1 15	1 16	1 20	1 23	1 25	1 32	1 37	1 38	1 60	1 79	2 02
24 hrs	1 18	1 15	1 16	1 24	—	1 27	1 39	1 42	—	1 76	1 89	2 14
72 hrs	1 19	1 18	1 20	1 28	1 32	1 35	1 42	1 48	1 55	1 91	2 00	2 28

Immediate	2 11	2 25	2 92	3 10
24 hrs	—	2 71	3 21	3 39
72 hrs	2 81	2 88	3 31	3 48

The determinations were made at room temperature, varying between 15°C and 27°C   To estimate the effect of temperature on the pH, determinations were made at 21°C and at 37°C on a number of samples, the potentiometer being standardized at each temperature with an M/20 solution of sodium hydrogen phthalate of pH 3 97   The figures show that there is a small decrease in pH with an increase in temperature —

Temperature   pH of samples

21°C	1 20	1 21	1 21	1 30	1 30	1 39	1 47	1 50	2 00	2 02	2 32	2 86
37°C	1 16	1 19	1 19	1 21	1 28	1 34	1 42	1 42	1 94	1 99	2 32	2 85

21°C	2 94	3 34	3 47
37°C	2 92	3 31	3 44

Sources of error from sampling

In order to interpret the variations in acidity in successive samples of gastric juices, it is necessary to know how far these changes occur in the stomach as a whole, and how far they may be accounted for by local variation , in other words, information is required about the degree of mixing of the stomach contents   This information was obtained by removing samples simultaneously from two tubes, the inlet holes of which were separated by varying distances

In one group of experiments a tube was placed in the body of the stomach and another in the pyloric antrum, and samples removed simultaneously during varying periods in which food was eaten, and also during the fasting period at night   The position of the tubes was verified by X-rays   Tests of this kind were made on 6 cases of duodenal ulcer, one

of gastric ulcer, and on 2 normal subjects   (Fig 3)   They show that the
only systematic discrepancies in the acidities of these two parts of the stomach
occur after meals, immediately following which there is a considerable
neutralization of the proximal end of the stomach, whereas the pyloric
antrum is affected to a lesser extent, or not at all   later, the acidities
approximate more or less rapidly   During the fasting period the acidity
of the stomach is fairly uniform   Patients with gastric and duodenal ulcer,
and normal subjects, all behave similarly in these respects
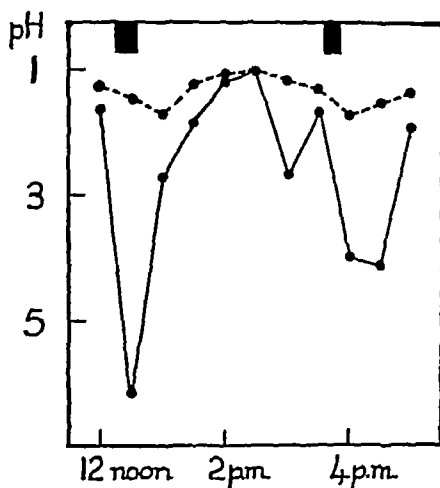
Fig 3   S G , male, age 42, duodenal ulcer, 27 5 47   Shows the changes in acidity in the body
    of the stomach (continuous line) and in the pyloric antrum (broken line), recorded
    simultaneously over a 5 hour period during which two meals, marked by black rectangles,
    were eaten   In this, as in subsequent diagrams, the pH scale is inverted so that acidity
    increases upwards along the ordinate

Secondly, samples were taken from two tubes, both in the body of the
stomach, but with their holes separated by 3–4″ , two patients with duodenal
ulcer and one normal subject were tested in this way   (Fig 4)   This
showed that quite large variations in acidity might exist between points
separated to this extent, but that the systematic differences after meals
were *no longer found*

Thirdly, in two normal subjects, samples were taken from two tubes
lying alongside with their holes adjacent   The differences were small, but
occasionally substantial

To find out how great was the effect of the mixing inherent in removing
the sample, one observation was made in which two specimens of juice
were removed successively through the same tube, as rapidly as they could
be obtained   This showed that two such samples might differ considerably
in acidity, especially if the acidity were low, and that the second sample
might be more or less acid than the first   The largest discrepancy between
such samples where the acidity was greater than pH 2 was 0 22 pH units

Results

The result of each 24-hour test was represented as a curve, obtained by plotting the acidity of each sample against time   The pH scale was inverted so that acidity increased upwards along the ordinate

The general shape and features of these curves will be first described Subsequently they will be analysed in respect of particular features
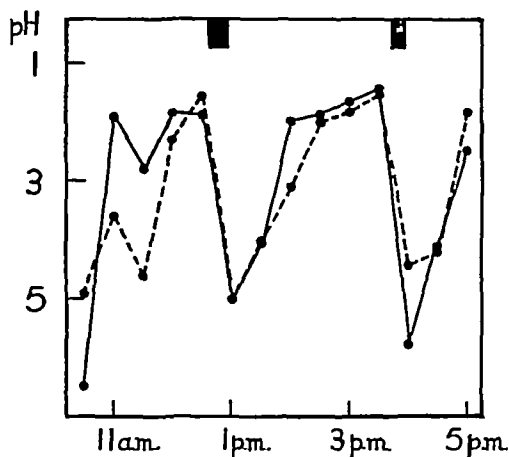
Fig 4   A J , male, age 26, normal, 18 6 47   Shows the changes in acidity in the body of the stomach recorded from a tube high in the body (continuous line), and from another 3 inches lower (broken line), over a 6 hour period during which two meals, represented by black rectangles, were eaten

Subjects without ulcer (20 cases)   During the day the chief factor affecting gastric acidity is the two-hourly ingestion of food or milk which is followed by a fall   The degree of neutralization recorded is dependent greatly on the mixing and sampling of the stomach content   Subsequently the acidity rises, tending to reach a maximum before the next meal   After the last milk at 10 p m the acidity again rises towards a maximum at about midnight Thereafter, during the hours of sleep, normal subjects display much variation in the behaviour of intragastric acidity (Fig 5), particularly after food is no longer present in the samples, an event usually occurring between 1 and 3 a m   In some subjects the acidity during this period remains nearly as high as earlier in the night   (Fig 6) In others it falls, usually to between pH 3 and 5, but in 3 cases it fell to neutrality   In one subject without ulcer the acidity never rose above pH 4 5 (achlorhydria)

Duodenal ulcer (20 cases)   Two curves determined on different occasions in a patient representative of this group are shown in Fig 7, and all the curves between 8 p m and 6 a m in Fig 5   In comparison with normal subjects it is usual to observe a less conspicuous neutralization after food and a higher acidity during the period of the night in which food is not found in the gastric samples   Curves of this type were found in all but 3 of the cases of duodenal ulcer tested, although the maximum acidity was not always as high as in the examples shown
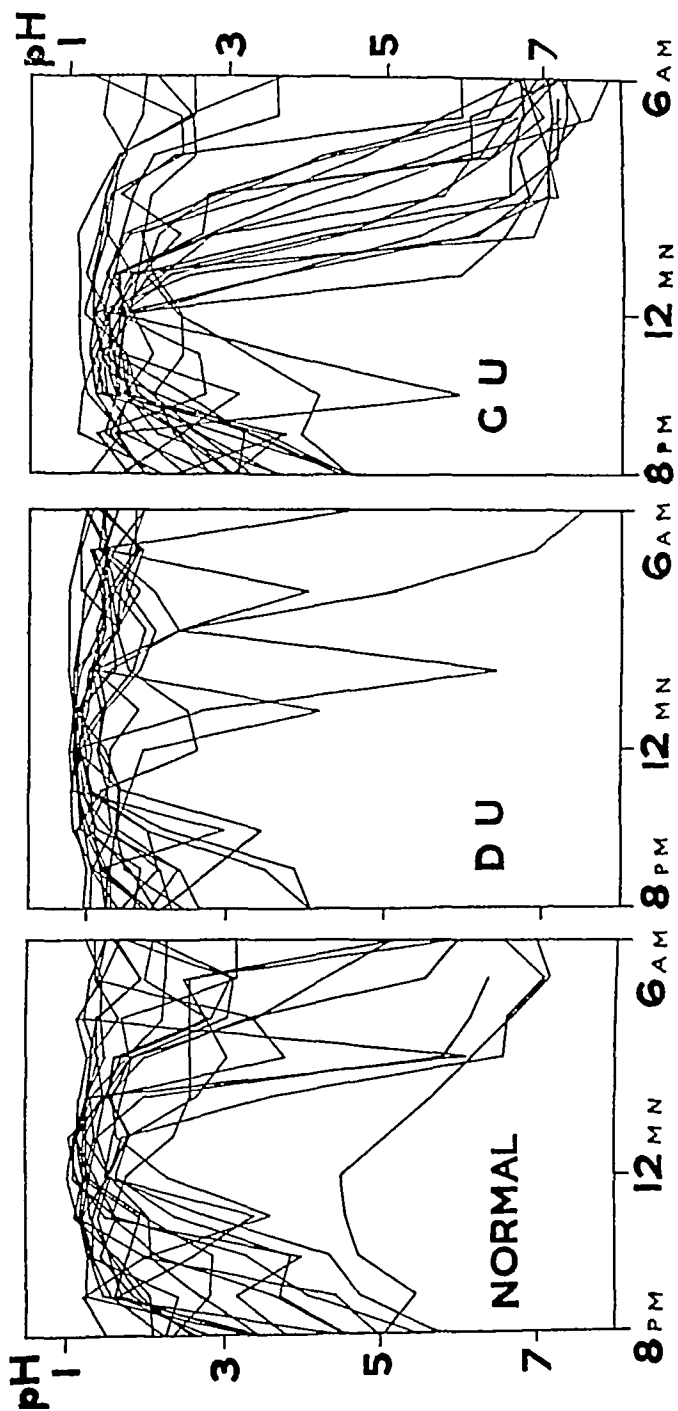
Fig 5   Shows the level of acidity in the stomach at intervals of one hour, during the night only (from 8 p m 6 a m), in all subjects in each group

In two of the exceptional cases the acidity fell to neutral during the night, and in the third to pH 4   In the first case tests were done in duplicate, and on each occasion, when food disappeared from the samples during the night, the acidity fell to neutral in a manner similar to that found in most
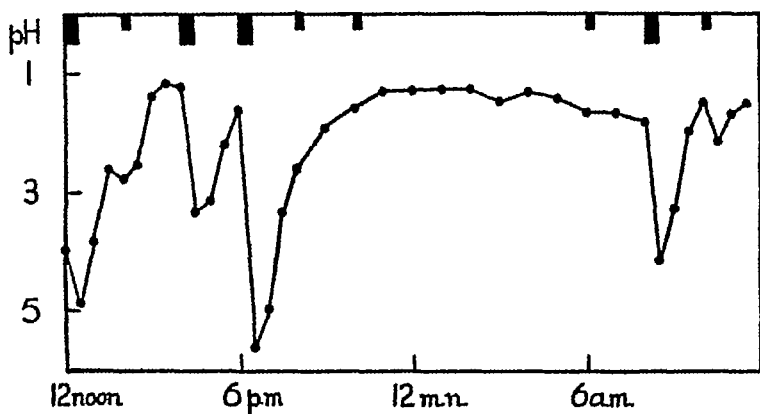
Fig 6   E F , male, age 36, normal, 26 5 48   Shows the changes in acidity throughout 24 hours   In this, as in the two subsequent diagrams, the large black rectangles represent meals and the small ones drinks of milk

cases of gastric ulcer   The diagnosis of duodenal ulcer was confirmed at operation on this patient, who was one of the two women in the duodenal series   In the second case the fall to neutral occurred at 2 a m , 2 hours after food had disappeared from the samples, and was accompanied by the
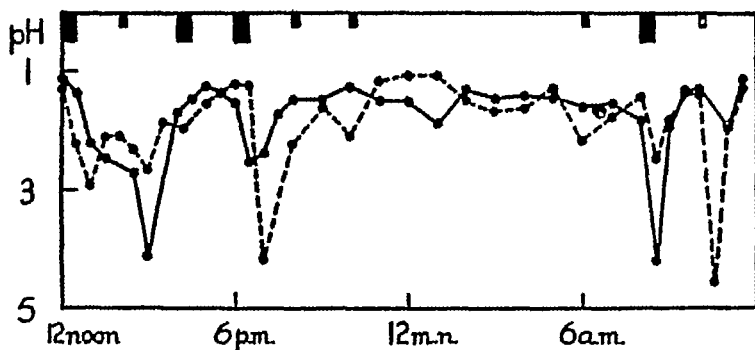
Fig 7   P M , male, age 45, duodenal ulcer   Shows the changes in acidity throughout 24 hours on each of two occasions (continuous line, 10 2 47 , broken line, 17 2 47)

appearance of bile   The acidity immediately rose again and the bile disappeared   This occurrence we attributed to a large regurgitation through the pylorus

Gastric ulcer (23 cases) As will be seen from Fig 5, the curves from patients with gastric ulcer are, broadly speaking, of 2 types In the more usual type, displayed by 16 patients (*see* Fig 8), gastric acidity fell to neutral or slightly to the alkaline side when food disappeared from the samples The acidity remained low until after the early morning milk During the day the maximum acidity tended to be lower than in normal subjects, than in those with duodenal ulcer, and those subjects with gastric ulcer displaying the second type of curve Cases of this second type, found in 7 subjects, showed a maximum acidity comparable with the normal During the night the acidity was maintained above pH 3 7, even when the samples contained no food , thus the distribution of curves in this period compared very closely with the normal During the day it was noticeable that the
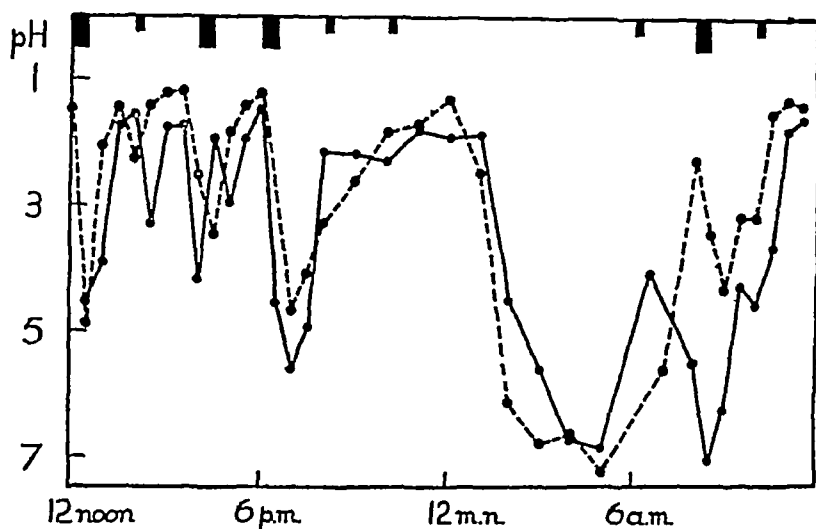
Fig 8 E B , male, age 42, gastric ulcer Shows the changes in acidity throughout 24 hours on each of two occasions (continuous line, 8 4 47 broken line, 23 4 47)

degree of neutralization following ingestion of food tended, in this group, to be less than normal In one of these cases only the 5 a m and 6 a m specimens contained no food, suggesting that the maintenance of the acidity throughout the night in this case might be due to delayed emptying, in contrast to the other 6 exceptional cases where emptying took place at the usual time, namely between 1 and 3 a m

The common type of curve, characterised by neutralization of the stomach at night and a subnormal maximum acidity during the day is hereinafter referred to as the majority type of curve The less common response in which the acidity is maintained at night and in which the reduction in acidity following meals is less than normal is called the minority type It is not asserted that a particular patient will always show the same type of curve

Comparison of the results

In order to see to what extent particular characteristics are peculiar to gastric or duodenal ulcer, the results have been analysed for the following features —

1   *Maximum acidity*   The mean of the 3 most acid samples in each test has been used to compare the subjects with respect to maximum acidity   Chiefly for ease of comparison with the results of other workers, the pH values were converted to mEq/l by the curve shown in Fig 2, and then averaged   When more than one test was done on a single subject, the figures for the separate tests were averaged
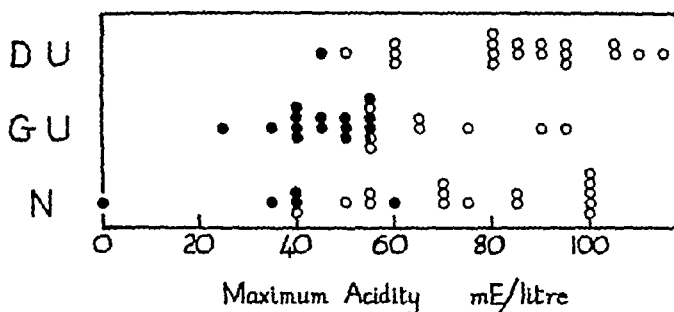
Fig 9   Shows the maximum acidity to the nearest 5 mEq/l reached by each subject in the 24 hour tests *(see text for details)*   In this, as in the subsequent diagrams, those cases where the acidity fell below pH 4 in two successive samples between midnight and 6 a m are represented by a black disc, and those in which the acidity remained above this level by an open circle   The one case in which the behaviour of the acidity differed in this respect on different occasions is represented by a half filled circle

The results are shown in Fig 9, each dot or circle referring to one subject   It will be seen that the majority of patients with gastric and duodenal ulcer have maximum acidities within the normal range   There is a slight tendency for the duodenal ulcers to be distributed on the more acid side of normal, and for the gastric ulcers to be distributed on the less acid side   This is no more than a tendency, for the mean values are for normal subjects (66 3 mEq/l), for duodenal ulcer (83 1 mEq) and for gastric ulcer (53 5 mEq)   The difference between duodenal ulcer and normal is significant, but ceases to be so if the patient with achlorhydria is excluded (as we think he should be) in calculating the mean and standard errors of the normal group   (P = 0 07) *(see* Table I)   The difference between gastric ulcer and normal is not significant

In Fig 9, and in the subsequent diagrams (Figs 10–13 inclusive), cases having 2 consecutive specimens less acid than pH 4 between 12 midnight and 6 a m have been represented by a black dot, and those which remained more acid than this by a clear circle   It will be seen that individuals who maintain a moderate acidity throughout the night tend to reach a higher acidity at the peak periods   This is particularly noticeable in the gastric

ulcer group, where the mean maximum acidity for the majority group is
45 6 mEq/l (significantly less than normal), and for the minority group
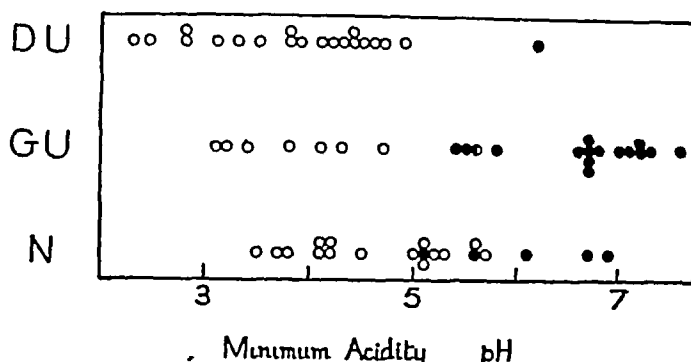71 6 mEq/l

. Minimum Acidity   pH

Fig 10   Shows the minimum acidity (to the nearest 0 1 pH unit) reached by each subject in
the 24 hour tests (*see text for details*)   The cases are differentiated as before according
to their behaviour at night

2   *Minimum acidity*   The pH values for the three least acid samples
were averaged for each subject in the way just described , the mean values
are plotted in Fig 10   The pH scale is adhered to in this instance because
it would be difficult to represent graphically or compare statistically the
distributions which result from conversion to a linear scale

Duration of acidity exceeding pH 2   Hours
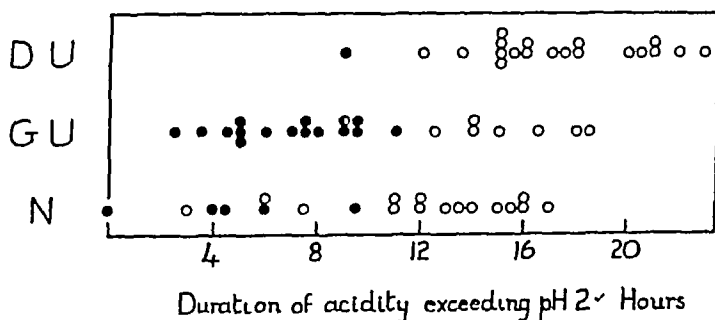
Fig 11   Shows the number of hours out of the 24 during which the acidity exceeds pH 2 (to
the nearest ¼ hour)   As before, each disc or circle represents a single subject, distinguished
according to their acidity at night

The distribution of values in each group overlap considerably   The
means are pH 4 97 for the normal group, pH 3 90 for duodenal ulcer, and
pH 5 76 for gastric ulcer   Subdividing gastric ulcer we find means of pH
6 61 for the majority group, and pH 3 80 for the minority group   For the
entire groups the figures for duodenal ulcer differ significantly from normal ,
those for gastric ulcer do not , the majority and minority groups of gastric
ulcer differ significantly from normal

3   *Duration of high acidity*   From each curve was estimated to the nearest half hour the number of hours of the 24 during which the acidity exceeded pH 2, the results being plotted in Fig 11

Duration of acidity less than pH 5 5   hours
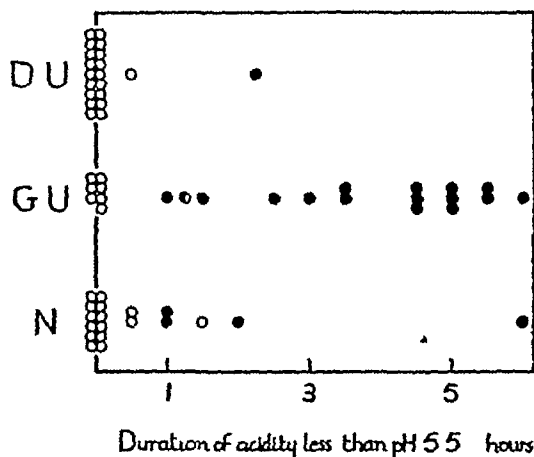
Fig 12   Shows the number of hours out of the 24 during which the acidity was less than pH 5 5 (to the nearest ¼ hour)   Notation as in previous figures   The patient with achlorhydria has been excluded from the normal group

The mean duration for duodenal ulcer, 17 0 hours, is significantly greater than that of the normal group (10 3 hours)   The mean duration for gastric ulcer, 9 5 hours, is not significantly different from normal , the means of the majority group of gastric ulcer, 6 8 hours, and of the minority group, 15 5 hours, are significantly less and greater than normal respectively
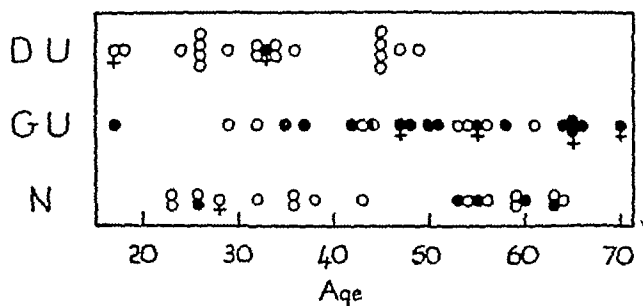
Fig 13   Shows the age of each subject   Women are represented by the addition of a cross to the dot or circle   Notation as before

4   *Duration of low acidity*   In a similar manner, the number of hours was counted during which the acidity was less than pH 5 5, the results being shown in Fig 12   In the duodenal group an acidity as low as this was only reached in 2 cases   In the normal group, one case, an achlorhydriac, is omitted from the diagram, having a duration of 13¼ hours below this level

TABLE I

Levels of significance of differences between observed characteristics in normal, gastric, and duodenal ulcer groups

	Duodenal ulcer and Normal		Duodenal ulcer and Normal excluding patient with achlorhydria		Gastric ulcer, majority group, and Normal		Gastric ulcer, minority group, and Normal		Gastric ulcer, entire group, and Normal	
	t	P	t	P	t	P	t	P	t	P
Maximum acidity mEq/l	2 21	<0 05	1 87	0 07	3 19	<0 01	0 62	0 55	1 80	0 08
Minimum acidity pH	2 43	<0 02	—	—	0 56	<0 001	3 54	<0 01	1 58	0 11
Duration of acidity exceeding pH 2	5 10	<0 001	—	—	3 47	<0 01	3 72	<0 001	1 68	0 1
Duration of acidity less than pH 5 5 Number of cases with duration of 1 hour or more	χ^2 4 32	<0 05	—	—	χ^2 18 3	<0 001	χ^2 2 7	0 1	χ^2 0 72	<0 01

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of acidity   Only 3 other normal subjects were below this level for more than one hour, whereas in the gastric group, those so behaving were in a majority of 16 to 7

Table I gives the levels of significance of the differences between the mean values of these characteristics for each group   In the case of maximum acidity, minimum acidity and duration of acidity exceeding pH 2, the standard errors have been calculated assuming the values to be normally distributed In the case of duration of acidity less than pH 5 5, so many individuals have values of zero that the numbers of cases exceeding or not exceeding an arbitrary value have been tabulated in a 2×2 contingency table and χ^2 calculated

Duplicate observations

Tests were made on different occasions to see whether the stomach varied in its behaviour from day to day   The duplicate tests were separated by periods varying from 3 days to 3 weeks   Complete 24-hour tests were carried out in duplicate on 4 cases of duodenal ulcer and 2 cases of gastric ulcer   In 2 additional cases of gastric ulcer, samples were taken for a 12-hour period at night in addition to the 24-hour test   Examples of duplicate tests are shown in Figs 7 and 8   It will be seen that the main features of the curves are reproduced in each case   The quantitative differences between duplicates are shown below under the same headings as those under which the summarized results were presented   The 2 partially duplicated tests on cases of gastric ulcer are included in the maximum and minimum acidity tables, but not in the other two

1   *Maximum acidity*

The pairs of maxima (average of 3 highest as hydrogen ion concentration in mEq/l) are as follows —

	Duodenal ulcer				Gastric ulcer			
1st test	80	91	113	35	14	35	66	76
2nd test	93	99	108	53	35	70	48	83

2   *Minimum acidity*

The averages of the 3 least acid samples in pH units for each pair of duplicates are as follows —

	Duodenal ulcer				Gastric ulcer			
1st test	3 06	4 28	3 47	6 02	6 61	6 89	4 70	3 09
2nd test	3 92	3 90	3 16	6 48	6 78	6 53	6 44	4 16

3   *Number of hours in which acidity exceeds pH 2*

	Duodenal ulcer				Gastric ulcer	
1st test	19	$10\frac{1}{2}$	16	$9\frac{1}{2}$	0	$4\frac{1}{2}$
2nd test	17	19	$14\frac{1}{2}$	$8\frac{1}{2}$	$5\frac{1}{2}$	8

4   *Number of hours in which acidity is less than pH 5 5*

	Duodenal ulcer				Gastric ulcer	
1st test	0	0	0	2	5	4
2nd test	0	0	0	2½	5	5

In describing our results in patients with gastric ulcer, the curves were divided into 2 types   In 4 of these patients the relevant parts of the tests, namely 8 p m till 8 a m , were repeated   Two patients of the first group and one of the second showed curves of similar type on the second occasion The remaining case showed curves of the first type in one, and of the second type on the other occasion

Effect of preceding alkali therapy

There is evidence that a "rebound" secretion of gastric juice follows the ingestion of absorbable alkali such as sodium bicarbonate (1)   The possibility therefore exists that any difference between subjects with and without ulcer is due to preceding treatment with alkali

To investigate this samples were obtained in the usual way from 8 patients with duodenal ulcer and 5 with gastric ulcer, who had been treated with alkali up to the day before the test, and from 8 patients with duodenal ulcer and 4 with gastric ulcer who had not received alkali   No alkali was of course given during the test   Since the duration of acidity exceeding pH 2 was the quantity in which patients with duodenal ulcer differed most conspicuously from normal this quantity was chosen for analysis of any possible effect of alkali   For duodenal ulcer the mean duration for those previously treated with absorbable alkali was 18 hours, and those who had not received alkali 17 hours   For gastric ulcer the mean for those who had had alkali previously was 11 hours, and for those who had not had alkali 8 hours   The scatter in each group is large, and renders the differences insignificant

In 2 cases tests were done after periods of 10 and 13 days when no powder was given, and again after 8 and 7 days during which powder had been administered 4 times daily, the last dose being given 4 hours before the tests began   The differences between the curves are in neither case greater than might be ascribed to chance variation

Age

It is possible that any difference between the groups is entirely dependent on differences in the ages of the patients in each group   Fig 13 shows the age distribution of the patients in each group   Women are indicated by the addition of a cross to the dot or circle   It will be seen that the age incidence of the patients with gastric ulcer is higher than those with duodenal ulcer, and that that of the normal series covers almost the whole range

It is possible that the ability to maintain the acidity of the stomach during the night is lost with advancing age, and that the frequency with which this ability is lost in cases of gastric ulcer is due to the tendency of this lesion to occur in older people

In Fig 13, as before, those cases which remain acid at night are indicated by a circle, and those which become neutral by a black dot It will be seen that there is a slight tendency for the black dots in the normal group to occur at the upper end of the age scale, but that in the gastric ulcer group there is no such tendency There is, therefore, no evidence that the difference in secretory behaviour can be ascribed to age

There is no correlation in any group between age and maximum acidity The correlation coefficients are $+0\ 34$ for duodenal ulcer, $-0\ 16$ for gastric ulcer, and $-0\ 12$ for the normal group None of these is significant

Significance of the changes in acidity at night

To investigate further the mechanism by which the changes in acidity in the stomach are produced, a limited number of observations were made in which continuous suction was applied to the stomach throughout the night, the total quantity obtained during each successive half hour being collected separately, and the volume and pH measured These aspirations were personally supervised, and repeated clearing with air injected into the tube was found to be necessary It is our opinion that observations on continuous aspiration, in which these precautions have not been taken, must be viewed with grave suspicion The last meal was given at 6 p m At about 9 30 p m a Ryle's tube was passed, and all available fluid was aspirated from the stomach A drink of 200 ml of Ovaltine was then given, and aspiration commenced 15 minutes later Traces of the Ovaltine were present in the aspirate for $1\frac{1}{2}$–2 hours after beginning the test, after which the juice was clear, although sometimes bile-stained The aspiration was continued until between 7 and 8 a m , after which food and drink was allowed

A test of this kind was carried out in 2 cases of duodenal ulcer and 5 cases of gastric ulcer Two examples are shown in Figs 14 and 15, in which the continuous line with black dots shows acidity in mEq/l and the broken line volume of aspirate in ml /min for each half hour throughout the night Also shown for comparison are the relevant portions of the 24-hour sampling test which had been carried out on another occasion (line with clear circles)

The rate at which fluid was aspirated was at first high in all patients , the acidity was also high after the neutralizing effect of the Ovaltine was overcome In the example of duodenal ulcer shown (Fig 14), it will be seen that secretion of acid juice continued throughout the night, but that there was a tendency for both acidity and volume to fall as time passed The other case of duodenal ulcer behaved similarly

In the case of gastric ulcer shown (Fig 15), it is clear that secretion ceases when food is no longer present, the acidity falling to zero, and the volume to low levels   That the cessation occurs several hours earlier than the drop in acidity in the sampling curve, may be attributed to food being more rapidly removed by aspiration than by normal gastric emptying   In 2 other cases of gastric ulcer, in which the stomach contents became neutral or nearly so in the early morning, the volume of fluid that could be withdrawn by continuous aspiration from the stomach was small during this period
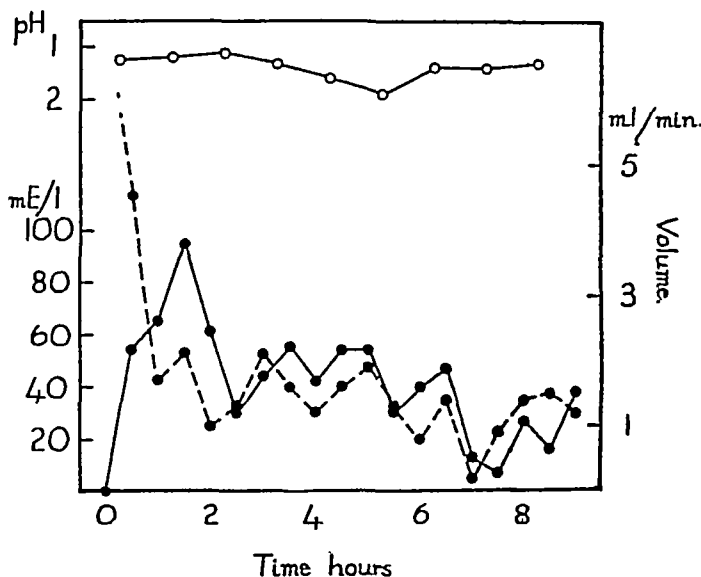
Fig 14   S L , male, age 32, duodenal ulcer   The two lower curves represent the result of a continuous aspiration experiment (11 10 47)   The acidity (in mEq/l) of the ½-hourly aspirate is represented by the continuous line, and the volume (in ml /min) by the broken line   The observation was begun at 10 10 p m   The upper curve (open circles) shows the intragastric acidity over the same period of the 24 hours as determined on another occasion (7 10 47)

These observations thus suggest that the reason why certain stomachs, notably those with duodenal ulcer, remain highly acid throughout the night, is that the stomach continues to secrete a highly acid juice, and that the reason why certain stomachs approach neutrality when food has gone, is because the secretion of acid has stopped   That this is the explanation, rather than neutralization by duodenal regurgitation or secretion of buffers by the stomach, is suggested by two other pieces of evidence

Firstly, bile was present during the period of neutrality in only 10 out of the 16 cases in which neutralization occurred, and frequently the fall in acidity took place several hours before bile appeared   Table II shows the number of bile-stained samples occurring during the night (8 p m —8 a m) in each group, the gastric ulcer series being divided into 2 groups according to the behaviour of the acidity at night   It shows that absence of bile-stained samples is commonest in duodenal ulcer, and that bile-staining is

less common in the cases of gastric ulcer which become neutral at night than in those that remain acid   We have twice observed regurgitation of duodenal contents sufficient to cause almost complete but transient neutralization of the stomach contents
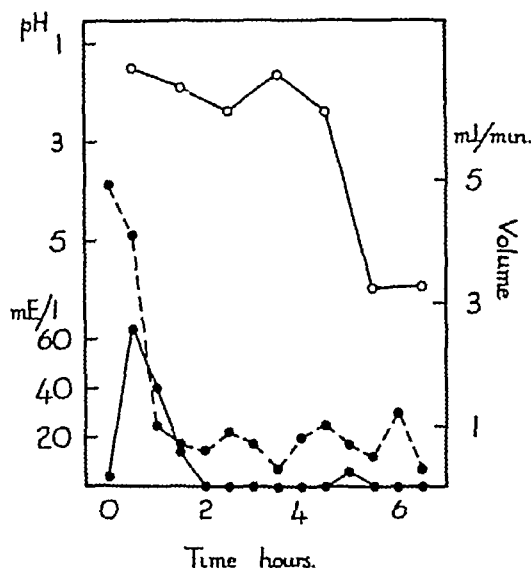

Fig 15   F W , male, age 51, gastric ulcer   Shows the results of a continuous aspiration observation (11 3 48) and of intermittent sampling (10 3 48)   Notation as in Fig 14   The time scale begins at 11 0 p m

If, finally, the drop in acidity were due to buffering of acid, it should be possible to demonstrate an increase in titratable buffer after the drop has occurred   In one case the buffer concentration was determined by measuring the quantity of N/10 NaOH required to convert the pH of 3 ml of the gastric juice from 2 5 to 6 0   The sample was brought to pH 2 5 by adding 1 0 N HCl or NaOH as necessary   The amount of titratable buffer tended to fall, rather than rise, when neutralization occurred, in contrast to the rise in buffer after a meal

It is possible that the ability to secrete acid gastric juice in the absence of a food stimulus is destroyed by ulceration of the gastric mucosa   We have not investigated the behaviour of the stomach after proved healing of the ulcer, but the 7 cases of gastric ulcer in which secretion of acid continued all night included some with very large craters, and some in whom symptoms had persisted for many years   The occasional occurrence of secretory behaviour of this kind in people who had never had digestive symptoms suggests also that it is not a sequela of ulceration

The occurrence of a minority of cases of gastric ulcer in which secretion continues through the night is open to two interpretations   It might be that patients with gastric ulcer are divisible into two groups, one of which

regularly becomes neutral in the fasting period, the other consistently remaining acid , alternatively, all the cases might behave in the same way, each case becoming neutral on some days and remaining acid on others in the approximate proportion of two to one   In the latter case, agreement of duplicate tests in this respect would be fortuitous   In favour of the first interpretation, the two types of curve found in gastric ulcer differ not

TABLE II

Shows the incidence of bile stained samples between 8 p m and 8 a m

	None	One or more
Duodenal ulcer	14	6
Gastric ulcer majority	6	10
Gastric ulcer minority	1	6
Normal	8	12

only in the minimum acidity reached, but also in the maximum acidity achieved during the peak periods, so that the difference in behaviour is extended over the whole 24 hours   In favour of the second is the single failure to duplicate the drop in acidity, which suggests that both types of behaviour can occasionally occur in a single stomach   We have not a sufficient series of duplicates to decide with certainty between the alternative hypotheses

DISCUSSION

Two methods have been used previously to investigate gastric acidity The test meal gives information concerning the changes that occur after a fixed period (usually one hour) or over a period of up to 3 hours (fractional test meal) after ingesting a standard meal   The meals chosen are in general by no means representative of what is normally eaten, and the time period is short   Palmer (12) has shown, and we have confirmed, that patients with low acidity to the fractional test meal may yet have abundant acid in their stomachs at certain periods when eating ordinary food   It is clear that information derived from studies of this kind are of limited value in assessing the place of acid in the genesis of ulcer   Nevertheless, the method has yielded results that agree with ours in suggesting that most patients with duodenal ulcer respond to a given stimulus with an increased secretion of acid, while most patients with gastric ulcer do not

Ryle and Bennett (14) investigated 100 normal medical students by the fractional test meal technique, using a pint of gruel as a food stimulus, and found that the maximum acidity lay between 10 and 45 mEq per l in 80%   Hunter (6) found that in all of 8 cases of duodenal ulcer similarly investigated maximum gastric acidity exceeded 45 mEq/l , and had a mean of 68 mEq/l   Vanzant and others (17, 18), withdrawing the stomach contents

one hour after ingesting 8 arrowroot cookies and 400 ml water, found that the mean acidity in 1495 males aged 20 to 40 with duodenal ulcer was 60 mEq/l in contrast to 45–50 mEq/l in male subjects of similar age in whom no ulcer had been found

It will be noted that the figures for maximum acidity obtained here are consistently higher than those obtained after test meals, probably because of the use of a less artificial and more potent food stimulus, but that the increase in the mean value for maximum acidity in duodenal ulcer (17 mEq/l) is of the same order

The second method that has been used is that of nocturnal aspiration Winkelstein (21) gave a standard meal at 4 30 p m and aspirated the stomach (presumably as completely as possible) at intervals of 2 hours throughout the night, starting at 7 p m By this means he compared 62 patients with duodenal ulcer and 23 patients with gastric ulcer, with 20 normal subjects It was not stated by what criteria the selections were made, nor is there information about the ages of the patients except that they were adult males The results are represented by a curve joining the mean acidities for each interval during the night the maximum level reached by this curve was 60 mEq/l for the patients with duodenal ulcer, 55 mEq/l for those with gastric ulcer, and 15 mEq/l for the normal group Free acid was stated to be absent in 9 normals, but in none of the ulcer patients The acidity fell slightly later in the night in the gastric ulcer cases, hardly at all in duodenal ulcer Voegtlin (19) removed small samples of gastric juice at intervals of 2 hours during the night, no food being eaten on the previous day He compared 53 proved cases of duodenal ulcer with 15 normal subjects of unstated age who were in hospital for investigation of seasickness He found no significant difference between ulcer patients and normal in the levels of acidity reached, the maximum level being about 50 mEq/l Sandweiss and others (15) gave a standard meal of ordinary food at 6 p m and aspirated the stomach completely at midnight Thereafter they obtained juice by complete hourly aspiration or by continuous aspiration By this means they were unable to detect any difference in the volume or acidity of juice obtained from 38 college students and internes and from 29 patients with duodenal ulcer Levin and others (9) gave a liquid meal composed almost entirely of carbohydrate at 5 30 p m , at 8 p m the stomach was aspirated completely, and then continuous aspiration applied for the following 12 hours Although the fluid obtained during each hour was collected separately, only the total volume and acidity of the whole night's aspirate are given By this technique 32 patients with duodenal ulcer, 8 patients with gastric ulcer, and 33 subjects of comparable age known not to have any gastro-intestinal complaints were investigated The mean acidities were 61 mEq/l for the duodenal group, 12 mEq/l for the gastric ulcers, and 29 mEq/l for the normals, the mean total volumes being 1004 ml , 623 ml and 581 ml ,

respectively   It is not evident from their results whether, in the cases of gastric ulcer, acid was recovered from the stomach throughout the night or whether it behaved as in our subjects

It will be seen that most of these observations are similar to those reported at the beginning of this paper   Perhaps some confidence may be felt that most observers agree that more liquid and more acid can be recovered during the night from the stomachs of patients with duodenal ulcer than from those of normal subjects   Nevertheless, the objection that led us to abandon our own observations remains, that it is uncertain whether this means that a smaller proportion of fluid was lost through the pylorus in patients with duodenal ulcer , it is, of course, true that if this were the explanation it might have some as yet undetermined connection with the aetiology of duodenal ulcer

Another method of investigating the acidity of the resting stomach is that of Bloomfield and his colleagues (2, 13), in which the gastric contents are aspirated 12 hours after the last meal and then continuously till the successive 10-minute volumes are approximately constant , this rate is termed the basal secretion   By this means were investigated an unstated number of patients suffering from various diseases but without digestive symptoms, in a few of whom a small volume of fluid with no free acidity was obtained   Such a finding Polland and Bloomfield (13) called basal anacidity, and described it as " quite uncommon " in normal people   Basal anacidity was found in 3 out of 9 cases of gastric ulcer tested   In one case the gastric juice contained " no free acid, but large amounts of clear glairy mucus with the appearance and consistency of egg-white," which was precisely the appearance of the night samples in many of our cases of gastric ulcer   Although the pH of these samples was not recorded, it was almost certainly near to neutrality   It is not possible to calculate whether the incidence of " basal anacidity," as detected by this technique, is greater in gastric ulcer than in normal people, since its numerical incidence in the latter group is not given by Polland and Bloomfield (13)   If anacidity under the conditions of the " basal secretion test " is equivalent to the fall to neutrality which we have found during the night, then cessation of acid secretion was found less often in Bloomfield and French's (2) series of gastric ulcers than in ours, but the difference is barely significant ($P = 0 07$)   It may be that the difference is due to the different techniques used, but it is also possible that a repetition of our work in North America, where gastric ulcer is less common than in London, would give different results   Bloomfield and French (2) recorded the range of basal acidity as 0 to 95 mEq/l in gastric ulcer and 24 to 140 mEq in duodenal ulcer   They regarded these differences as suggesting that gastric and duodenal ulcer were predominantly different disorders

The observations described in this paper give a more complete account of the variations in intragastric acidity during the course of a whole day than has yet been obtained in comparable series of subjects with gastric and duodenal ulcers, and without ulcers. We may now proceed to discuss their bearing on the hypothesis that peptic ulcer is due to the action of a gastric juice that is abnormally acid in reaction

This hypothesis has arisen because it seems that peptic ulcer is due to the action of a factor or factors that tend to persist over many years, sometimes over the adult part of a lifetime, in a given individual. This factor is not simply a local abnormality of an isolated patch of mucosa, for gastric ulcers, when excised, recur, duodenal ulcers are multiple in 37% of cases, and when either is treated by operation involving anastomosis of stomach and jejunum, ulceration of the jejunum may develop. The recurrence of ulcer seems to depend on the presence of HCl in the gastric juice, for with anacidity ulcers never occur

Even if there is an agent in the gastric juice which is concerned in the pathogenesis of ulcer, it is unlikely that this is the only operative factor. For there is usually only one ulcer in the stomach, and in duodenal ulcer the stomach is rarely ulcerated, and it must be inferred that most of the mucous membrane exposed to the gastric juice is resistant to it. There must presumably be a local factor, though this may, as in the possible case of physical trauma, be quite transient in its action. Both the natural history and the histological appearances of peptic ulcer suggest that the ulcer is affected by the two opposed processes of tissue destruction and tissue repair, sometimes the one, sometimes the other being in the ascendancy. It seems quite likely, therefore, that if there is an agent in the gastric juice, it may be one to which the intact epithelium is relatively resistant, but to which cells of a different character are more susceptible. In so far as acidity is concerned in this process, the hydrogen ions themselves might act as an injurious agent, as they can do, or they might facilitate the action of another agent such as pepsin. In either case it would seem likely that the degree of acidity and the time period over which it acted would be the operative factors

From the results presented in this paper it seems quite clear that an abnormally high degree of intragastric acidity is not the cause of gastric ulcer, at least in the average case during the chronic phase. For, in so far as the behaviour of these patients differs from the normal, it is in the opposite direction. The patient with chronic gastric ulcer tends to achieve lower values at the peak periods of intragastric acidity, and to maintain the stomach contents at a reaction approaching neutrality for longer periods than does the subject without ulcer. This conclusion holds even if we consider gastric ulcer as a homogeneous group. As we have seen, the cases tend to be rather sharply divided into two groups. These conclusions apply even more strongly to the majority group, in which intragastric acidity is around neutral after food has left the stomach. In the minority

group, on the other hand, neutralization following meals tends to be less complete than normal, and acidity above pH 2 to be maintained for a longer time, but it is doubtful whether significant conclusions can be drawn from comparing a selected group with high acidities with an unselected group of normal subjects

It is, of course, conceivable that, at the very beginning, a gastric ulcer may be caused in part by an abnormally intense degree of gastric acidity, and that the continued presence of this ulcer leads to a secondary reduction in acidity   This hypothesis could best be tested by investigating in the manner described in this paper, a series of cases of gastric ulcer over a period of years from the earliest acute phase to the fully developed chronic ulcer A less satisfactory method, but the only one available to us at the present time, is to compare the findings in patients with a history of less than a year's duration with others with histories extending over 10 years or longer In Fig 16 the maximum acidity of cases of gastric ulcer, whose symptoms
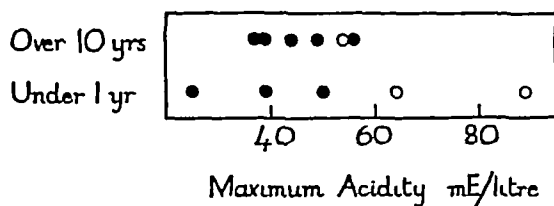
Fig 16   Shows the maximum acidity (average of 3 most acid samples) in 6 cases of gastric ulcer with symptoms for more than 10 years, and 5 cases of gastric ulcer who had had symptoms for less than a year   The cases are differentiated, as before, according to the behaviour of the acidity at night (*see* legend to Fig 9)

had lasted for not more than a year, is compared with those who had had symptoms for 10 years or more   Within the limits imposed by the small number of cases, no significant difference is demonstrated   The duration of symptoms has been similar in the two groups of patients with gastric ulcer presenting the different types of curves

Finally, it is conceivable that the relapses and remissions of gastric ulcer are associated with a waxing and waning of intragastric acidity   This point is so important that it requires far more careful investigation than we have been able to give in this paper   And while we have seen no evidence to support this idea, we have by no means sufficient to refute it

One of the most striking features of the gastric ulcer group has been the tendency, particularly in those forming the majority group, for the gastric contents to approach neutrality in the early morning   This phenomenon has not been previously observed, and indeed could not have been observed with the traditional method of titrating to two indicators turning at pH 3 and 9   It could only be detected by the electrometric method   Whether this peculiarity has anything to do with the pathogenesis of ulcer we have, at the present moment, no means of telling

In duodenal ulcer, on the other hand, we have observed that peak values of acidity tend to be higher (though the difference is not statistically significant), and high degrees of acidity tend to be maintained over longer periods of time than in normal subjects. Conversely, minimum acidities are not so low and periods of low acidity are not so prolonged as in normal subjects. Here, then, the hypothesis that an unusually acid gastric juice is an important factor in the pathogenesis of the ulcer is one that merits close consideration. The whole picture of the intragastric acidity in duodenal ulcer would seem to be that resulting from a high rate of secretion of acid gastric juice, and we have adduced evidence that this occurs, at least at night. The long duration of acidity over pH 2 and the higher minimum acidity are largely the reflection of the shorter and slighter neutralization of the gastric content by ingested food.

The obvious weakness of this hypothesis is the considerable overlap in the findings in duodenal ulcer and in normal subjects. It is not infrequent to encounter a subject who has never had (or who never remembers having had) ulcer symptoms, and who yet shows a higher degree of acidity than a subject with a long ulcer history. A little consideration shows, however, that such is to be expected even if the hypothesis were correct. In the first place, as we have seen, there is reason to believe that a local factor may be concerned in the pathogenesis of ulcer. It may be, therefore, that those people with highly acid gastric contents are susceptible to the development of duodenal ulcer, given the right circumstances, of which local injury to the duodenal mucosa may be one. Again, given this high degree of acidity, it is not unlikely that mucous membranes will differ from subject to subject in their resistance to it. Further, it has been seen that when the curves of gastric acidity are repeated under apparently similar environmental conditions, and with the ulcer in an apparently comparable state, some variation has been found. Finally, it is to be noted that our observations have been made when the patients were in bed in hospital and on a diet, conditions under which duodenal ulcers usually heal. Duodenal ulcers usually develop or recur where the patient is subjected to the stresses and strains of responsible work in a civilised society. It is not inconceivable that the abnormalities we have observed in the behaviour of intragastric acidity in patients with duodenal ulcer under hospital conditions would be enhanced under the more arduous circumstances of life outside. At the present time we do not see our way clear to putting the idea to the more rigid test of comparing intragastric acidities in patients with and without duodenal ulcer under the various circumstances of ordinary life. Wolf and Wolff (22) showed that in a subject without ulcer emotional stress and mental conflict led to a secretion of highly acid gastric juice ; but there is no evidence that patients with ulcer differ from normal in this respect

The second weakness of the hypothesis is the possibility that the changes observed in intragastric acidity in duodenal ulcer represent the effect of the ulcer rather than its cause   We cannot, by actual observation, refute this possibility, but we do not think it likely because we have not discovered any relationship between the intensity of the changes and the duration of symptoms   Consider, for example, the case of a male, aged 49, who had been admitted for hypertension, but who, 3 years previously, had suffered from attacks of epigastric pain relieved by alkali, for which he had been treated in another hospital, where a deformity of the duodenal cap had been found by Dr E Rohan Williams   He had had no symptoms and no treatment for two and a half years before his test was done, and yet his curve is typical of the duodenal group, remaining above pH 2 for 22 hours   This patient has been included in the duodenal ulcer series

It should be emphasised that the observations described in this paper in themselves provide no proof that acid is or is not a factor in the pathogenesis of ulcer   They have demonstrated that patients with duodenal ulcer do present rather high levels of gastric acidity for periods of the 24 hours that are significantly longer than normal when the subject is on a full and standard diet under hospital conditions   They are thus consistent with the acid hypothesis, and, taken in conjunction with the evidence drawn from animal experiment, and mentioned at the beginning of this paper, make the position of the hypothesis extremely strong as regards duodenal ulcer   But in gastric ulcer our observations have led to precisely opposite conclusions, which make it quite improbable that an abnormal degree of acidity acting over an abnormally long period of time is the chief agent in the causation of the lesion   As we have seen, some reservations have still to be made before this conclusion can be accepted as final   But the evidence is so strong that it is worth while considering briefly the chief implications

The first implication is that gastric and duodenal ulcer are essentially distinct disorders, since it seems probable that the chief agent in their pathogenesis is different   Others studying gastric acidity have made this suggestion before (2)   The idea receives independent support from considerations of the changing incidence of gastric and duodenal ulcer over the last half century (Jennings (8), Tidy (16), Morris and Titmuss (11), Craig (3)), and from post-mortem evidence   Thus, in a study of 4,000 consecutive post-mortems at Leeds, Stewart (7) found ulcers or the scars of healed ulcers in the stomach in 170 and in the duodenum in 230 cases   Of these, only 21 cases had an ulcer or scar in both stomach and duodenum, although this is double the number expected if the association were fortuitous   Twenty-two cases had more than one gastric lesion, as contrasted with 104 with more than one duodenal lesion   It seems to be relatively infrequent for a gastric ulcer to be complicated by another ulcer either in stomach or duodenum, but it is much more frequent for a duodenal ulcer to be complicated by another in the same division of the gut

These several considerations taken together lend strong support to the idea that in the majority of cases gastric ulcer is a disease with a pathogenesis distinct from duodenal ulcer   We say in the majority of cases, because it is by no means impossible that an abnormally high intragastric acidity may occasionally produce a gastric ulcer, though a duodenal ulcer would seem to be the more usual result

The second implication is that we have to look for something other than intragastric acidity as the essential agent in the pathogenesis of gastric ulcer   We have no new evidence to offer concerning this factor or factors   But those who are looking for it are urged to avoid what is probably a fallacy, namely that peptic ulcer can be conceived as a strictly homogeneous unit from the point of view of pathogenesis

Summary

1   Preliminary observations on continuous aspiration of gastric contents during the night showed a tendency for most subjects with duodenal ulcer to yield more, and most subjects with gastric ulcer to yield less hydrochloric acid per hour than normal subjects   The fraction of the gastric content aspirated was, however, unknown

2   To determine the range of intragastric acidity throughout 24 hours, samples were taken at half-hourly intervals by day and hourly intervals by night from an indwelling Ryle's tube whose tip was verified as lying in the body of the stomach   The subjects were in hospital and on a full and standard diet without alkali   pH was measured electrometrically

3   The sources of error of the method have been examined   Of these the most important is the sampling error, due to inadequate gastric mixing, particularly soon after food

4   Tests were made on 23 patients with gastric ulcer, 20 patients with duodenal ulcer, and 20 subjects without ulcer

5   In duodenal ulcer intragastric acidity differed from the normal in the following particulars —

  (a) The mean value for the maximum acidity (83 1 mEq per l) was greater than normal (66 3 mEq per l)   The difference was not statistically significant

  (b) The mean minimum acidity reached in the 24 hours was more acid than normal   The difference was statistically significant

  (c) The duration of high acidities (greater than pH 2) was increased   The difference was statistically significant

  (d) The acid rarely fell below pH 5 5 in either duodenal ulcer or normal, and no significant difference between the two was observed

  (e) The curves of intragastric acidity showed in general a higher level of acidity, a less conspicuous neutralization after food, and the maintenance of higher acidities at night after food had disappeared from the stomach

6   In gastric ulcer intragastric acidity differed from the normal in the following particulars —

(a) The mean value for the maximum acidity (53 5 mEq per l) was less than normal (66 3 mEq per l)   The difference was not statistically significant

(b) The duration of high acidities (greater than pH 2) was normal

(c) The duration of low acidities (less than pH 5 5) was increased The difference was statistically significant

(d) The most striking deviation from normal in the curves of intragastric acidity was the fall to, or near, neutrality during that part of the night when food had left the stomach, in 16 of 23 patients   In the remaining 7 this fall was not observed   Information is insufficient to determine whether this difference reflects a basic and consistent difference in secretory behaviour

7   Duplicate observations on individual patients have, on the whole, agreed well   The differences observed between the groups were not due to preceding alkali administration or the effects of age

8   Evidence is presented that in gastric ulcer neutralisation of gastric contents after food has left the stomach at night is due to cessation of acid secretion

9   The evidence here produced is quite consistent with the hypothesis that duodenal ulcer is caused by the action of abnormal degrees of acidity over abnormally long periods of time

10   The evidence is inconsistent with the hypothesis that gastric ulcer is due to the action of abnormal degrees of acidity acting over abnormally long periods

11   This and other evidence suggests that gastric and duodenal ulcer are diseases of different pathogenesis

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AN IMPROVED CALORIMETER FOR THE HAND

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DURING experiments in which a Stewart calorimeter (1) was employed for measuring the heat elimination from the hand, it became evident that the physical properties of this calorimeter are such that it is impossible to apply an accurate cooling correction   The Stewart calorimeter employed consisted of a cylindrical copper vessel containing 3 litres of water embedded in a large earthenware pot 1 4 cm thick, from which it was separated by a layer of granulated cork 3 5 cm thick at the sides, and 5 cm thick at the bottom

Such a calorimeter is thus lagged with material of considerable thermal capacity, in which complicated temperature gradients may be set up   The cooling rate at any particular moment depends not only on the temperature difference between the contents of the calorimeter and the surrounding air, but also on the recent history of temperature change in the calorimeter   The most recent temperature changes, of course, leave the biggest imprint on the temperature gradients in the cork, but remote temperature changes are not without effect   That the rate of cooling is in fact determined by previous temperature change was shown by an experiment, the results of which are shown in Fig 1   The calorimeter was filled with water at 29 372°C and the temperature observed every minute   At the end of 13 minutes, some of the water in the calorimeter was replaced by warmer water, bringing the calorimeter temperature to 33 180°C   Observations of the cooling rate were continued   Further replacements with cooler and warmer water were made subsequently   After each replacement there was a progressive change in cooling rate, which took many minutes to attain a steady value dependent on the temperature difference between the calorimeter contents and surroundings   The experiment imposed greater and more rapid temperature fluctuations than are ever met with during physiological observations, but the defects revealed are still present, though in less degree, under ordinary conditions of use

The cooling correction is not only impossible to compute accurately, but it is also large   The final steady value for the cooling corrections in the experiment quoted would be equivalent to a heat elimination of the order of 15 cals per minute per 100 mls of immersed hand   This is considerably greater than the heat elimination from a vasoconstricted hand, and about one-sixth of the heat elimination from a vasodilated hand.

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It was therefore desirable to have a calorimeter that —

  (1) required a very small correction for cooling, and

  (2) had walls of negligible thermal capacity

The calorimeter designed to possess these properties was constructed from a one gallon bucket type " Thermos " flask (Fig 2)   The normal lid was replaced by a square of thick plywood with an underlying layer of
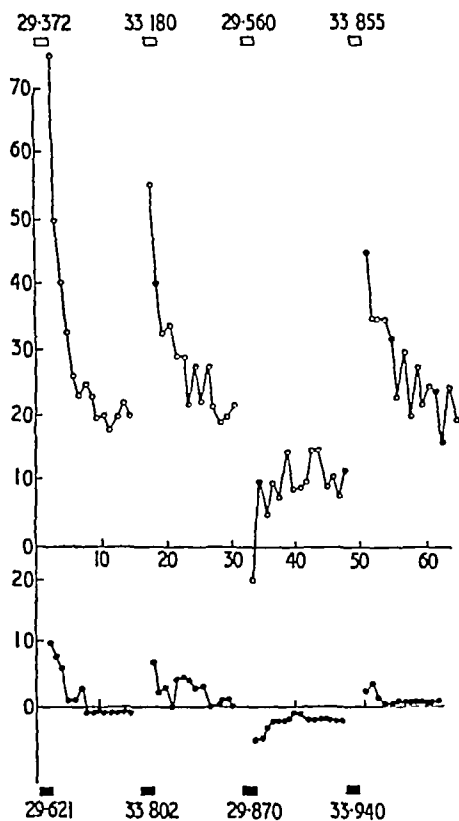

Fig 2

Fig 1

Fig 1   Cooling rates of the Stewart (upper curve, open circles) and Vacuum (lower curve, solid circles) calorimeters
Ordinate—fall in temperature per minute in thousandths of one degree centigrade
Abscissa—time in minutes

At the times indicated by the blocks, part of the water in the calorimeter was replaced by water at a different temperature   The figures adjacent to the blocks show the first thermometer reading after such a change   Room temperature, 21°C
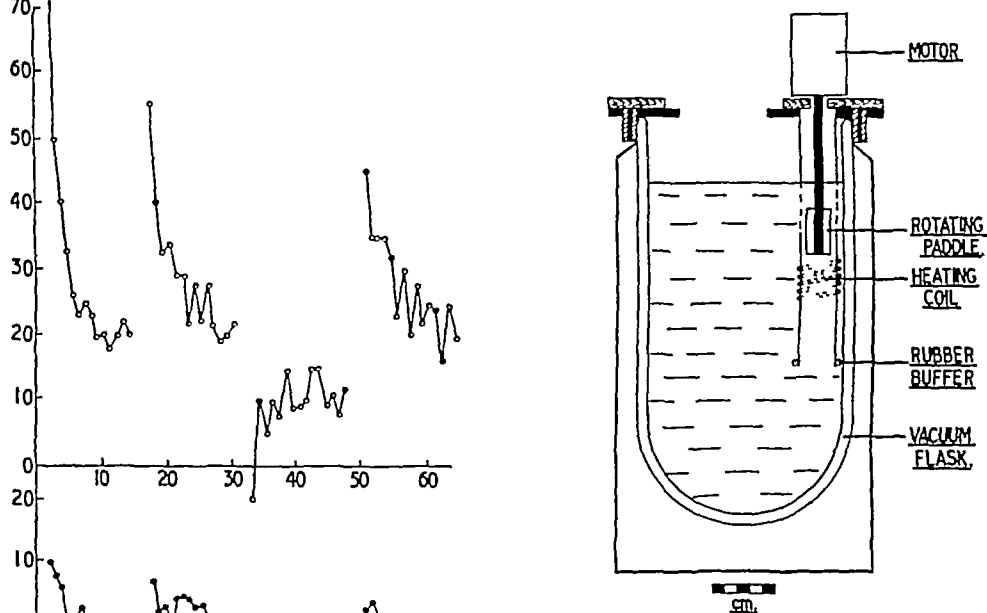
Fig 2   A sectional diagram of the vacuum calorimeter   The thermometer and its supporting rod do not appear in this section

6 mm sorbo rubber   The lid is held down by the clips forming part of the metal container, and guided into position by four rubber-covered prongs which engage with the outside of the rim of the vacuum flask (Fig 3)

The hand passes through a hole 8 cm in diameter cut in the plywood, and through an oval aperture loosely fitting the wrist cut in the sorbo rubber When in use during an experiment cotton wool is loosely wrapped round the wrist to occupy the space between it and the plywood
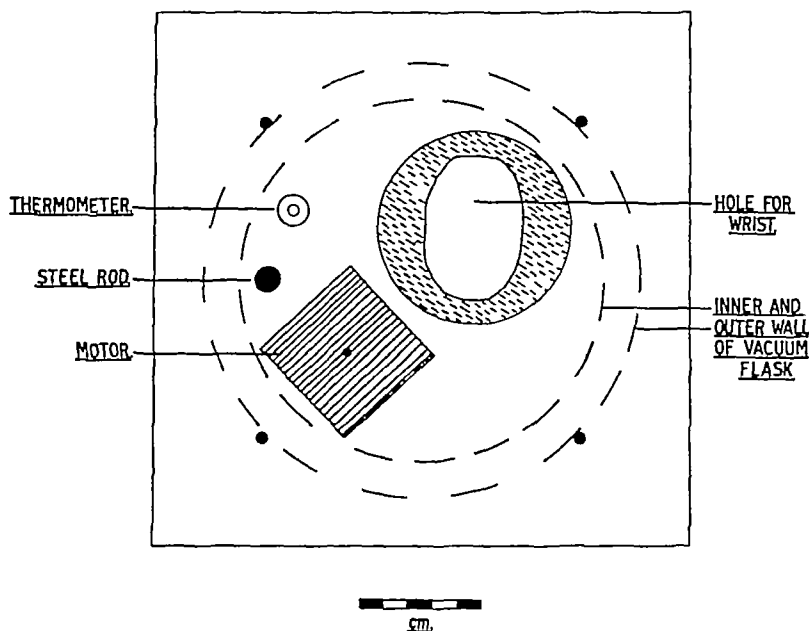
Fig 3   Plan of the lid of the vacuum calorimeter   The jar is exactly as received from the makers, all the attachments being made to the lid

The 3 5 litres of water filling the calorimeter is stirred by a flat paddle driven by a small electric motor which is mounted on a rubber seating attached to the top of the plywood   The paddle rotates in a copper tube 3 2 cm in diameter , water drawn into the tube from the lower part of the calorimeter is discharged by centrifugal force through a number of holes drilled in the tube below water level   That such an arrangement achieves efficient mixing is demonstrated by the rapidity of equilibration after the sudden addition of hot or cold water (Fig 1)

Energy is fed into the calorimeter at a constant rate by the stirrer However, under normal conditions of use, this is not quite sufficient to balance the loss of heat by cooling   A coil of resistance wire, insulated by woven glass impregnated with shellac, is therefore wound round the lower end of the copper tube and connected through a small external variable resistance with a 6 volt accumulator supplying the stirring motor   The value of the external resistance is adjusted by trial so that the calorimeter temperature does not vary by more than 0 01°C over successive ten minute periods when the contained water is 10°C above room temperature   After each experiment

the rate of heating or cooling of the calorimeter is observed over a ten minute period, the hole for the hand being closed with a sorbo rubber disc   The correction needed is seldom more than a few millidegrees per minute

The temperature of the calorimeter water is measured with a thermometer graduated in hundredths of a degree centigrade   This passes through a hole in the plywood and rubber, and is held at the top by a clamp attached to a vertical steel rod bolted to the plywood   The steel rod carries a travelling lens, focussed on the thermometer stem, with a block of thick perspex, ruled with a horizontal line on each side, interposed   The reading of the thermometer is made by bringing the top of the mercury and the two rulings into alignment, and then observing the scale reading   A small electric bulb, travelling with the lens provides adequate illumination

The water equivalent of each calorimeter must be individually determined   It is about 230 ml of water, and so is equivalent to about 6% of the contents

The measurement of the volume of hand immersed is probably the main source of inaccuracy in using the calorimeter, and considerable efforts have been made to reduce it   The following method is satisfactory   When the calorimeter has been filled and the lid placed in position, but before the hand or thermometer is inserted, a teated glass pipette, with a stem graduated in mms from the tip is inserted through the thermometer hole, and a " sounding " made of the distance of the water level below the top of the plywood board   This is done by advancing the teated pipette, gently pinching and releasing the teat and noting when an unbroken column of water first enters   The hand is placed in the calorimeter, and the subject made comfortable   A second sounding is made and an ink mark made on the ulnar border of the wrist at the level of the upper surface of the plywood board   This is watched by the observer during the experiment, and slight adjustments of the position of the hand are made to keep the mark in position   A final sounding is made at the conclusion of the experiment   The difference in water depth before and after immersion of the hand can be converted into volume of hand immersed by comparing with the alterations in depth resulting from adding measured volumes of water to the calorimeter   With a little experience, this method can be used to measure an added volume of water, and therefore an added volume of hand, with an error of not more than 10 ml (about 3%)

The results of an experiment, similar to that described for testing the Stewart calorimeter, are shown in Fig 1   It is evident that the whole system attains equilibrium within a much shorter time than the Stewart calorimeter   This provides, of course, an overall test of thermal hysteresis in the calorimeter walls, of efficiency of the stirring, and of the ability of the thermometer to record temperature differences   The magnitude of the allowance to be made for cooling of the calorimeter is very small, so that uncertainties about its exact value are correspondingly unimportant

SUMMARY

1   An improved hand calorimeter is described, based on a vacuum flask

2   Mechanical mixing ensures a constant energy feed into the calorimeter, and enables a single observer to measure heat elimination from both hands

3   Since heat is fed to the calorimeter at a rate approximately equal to that lost and the thermal insulation is extremely efficient, the cooling correction is very small   It is almost independent of the recent thermal history of the calorimeter, and can be determined with reasonable accuracy

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A COMPARISON OF METHODS FOR GAUGING THE BLOOD FLOW THROUGH THE HAND

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Introduction

IN certain experiments, in the course of which peripheral blood flow had been assessed in various ways, it became desirable to compare the results of a number of methods of gauging the blood flow through the hand over a wide range of rates of flow   The comparison was considered in two sets of circumstances ,   firstly, over periods of ten to twenty minutes when the peripheral circulation was maintained in as stable a condition as possible, although at widely different levels of volume flow, and secondly during short periods when rapid alterations in flow were occurring

Various methods of assessing blood flow were considered, such for example as the combined plethysmograph and calorimeter (7)   This enables the inflow of blood to be measured plethysmographically, or the dissipation of heat to the surroundings to be measured in a hand, the surface temperature of which is recorded but not controlled   It was thought, however, that in the first instance it would be more generally useful to employ well established methods and apparatus simultaneously in the two hands of subjects who had been demonstrated to have a symmetrical or nearly symmetrical circulation through the two hands when the hands were under identical conditions   Accordingly, the venous occlusion plethysmograph (4), hand calorimeter (16), and surface thermo-electric junction were employed

All three methods are open to criticism   With the plethysmograph it is uncertain that all veins draining blood from the hand are occluded so that the collection of blood in the parts distal to the cuff may be incomplete   Secondly, venous occlusion may itself alter flow   Errors due to these causes cannot be great because similar values for volume flow are obtained over a

wide range of collecting pressures and because, at low rates of flow, the volume increase is frequently linear over a considerable period   Thirdly, it is often difficult, particularly at high rates of flow, to determine the initial slope of the volume curve following application of the collecting pressure In the case of the calorimeter, uncertainties about the temperature of the arterial blood entering the hand (3, 7) and of the venous blood leaving it (11) make it impossible to convert figures for heat elimination into rates of flow (15)   Further objections to the calorimetric method inherent in the properties of the calorimeter are mentioned in more detail later   Finally, with regard to the surface thermocouple, it has never been supposed that a linear relationship exists between blood flow and surface temperature, which, in any case, depends upon many factors besides the blood flow through the part

In our experiments it was fully realised that the amount of blood flowing through the two hands might differ owing to the difference in posture and local temperature imposed by the methods employed   Although all the indications are that such changes are small the possibility of their occurrence must be borne in mind in assessing the results presented in this paper

Methods

Measurements were made on eight healthy males between the ages of 19 and 38   Each subject, wearing only short pants, sat on a well-upholstered seat inclined at an angle of 10 degrees to the horizontal, with a back extending above the head inclined at an angle of 75 degrees to the horizontal This was found to be a comfortable position even when maintained over periods of hours , it also facilitated the placing of the hands in the calorimeter and plethysmograph   The subject was in position for 30 min   before the observations were commenced

Experiments were performed from May to September   The room temperature on different days varied between 20°C and 24°C   Great care was taken to maintain quiet and avoid draughts

As the aim of the experiments was to make simultaneous comparisons of different methods, no observations of pulse rates or arterial blood-pressures were made and body temperature was not recorded

Plethysmograph technique   After preliminary experiments, the results of which are not reported here, a plethysmograph of the type described by Barcroft and Edholm (2) was used, with arrangements for continuously stirring the contained water   The plethysmograph was placed on an inclined plane   The water level in the vertical tube of the plethysmograph was slightly above a horizontal line through the sternal angle, in which position the veins of the hand were probably collapsed when the collecting cuff was not inflated   In this position a greater volume of blood can be accommodated in the hand before the rate of inflow falls off, thus considerably

increasing the accuracy with which measurements can be made   This position precluded immersion of the arm and plethysmograph in a water-bath, so a special tank was built around the body of the plethysmograph   It was found possible by adding ice, or water at varying temperatures, to this tank to maintain the water within the plethysmograph at any desired steady temperature between 6°C and 45°C with variations of not more than 0 5°C   The temperature could also be altered over this entire range within a few minutes

The hand was inserted into the plethysmograph as far as the radial styloid process   The collecting cuff, 4 5 cm wide and encircling the lower forearm, was applied as close to the plethysmograph as possible without causing an artefact on the volume curve when pressure was admitted to the cuff   In most experiments the lower edge of the cuff was about 4 cm above the radial styloid process   Unless otherwise stated the collecting pressure was 70 mm of mercury   When two plethysmographs were used simultaneously the collecting cuffs were supplied from a common air reservoir, all pipe connections to the two sides were of identical length and bore and the volume recorders were carefully matched (and on some occasions were interchanged during the course of an experiment)   The volume of the hand within the plethysmograph was determined at the end of the experiment by water displacement and the blood flow was calculated in terms of ml per 100 ml hand per min   Observations were made at the end of every minute and sometimes more frequently

Calorimetric technique   The arm and hand on the side of the body used for calorimetry hung almost vertically from the shoulder   The two Stewart calorimeters (16) used in most of the experiments were as nearly identical in dimensions and construction as possible   Each consisted of a copper can of water equivalent 200 ml holding 3 litres of water and manually stirred by raising and lowering a horseshoe-shaped stirrer through the depth of water about 24 times per minute   The can was surrounded by a 3 cm layer of cork chips enclosed in a large earthenware pot   The top was covered with 1 cm thick sorbo-rubber in which a hole was cut for insertion of the hand   The temperature of the water was measured with a mercury thermometer, graduated in hundredths of a degree centigrade   Before an experiment the hand and arm were held for 15 min in a water bath about 30°C and before the end of that time 3 litres of water from the bath were poured into the calorimeter   The hand was inserted and stirring was commenced without delay   No observations were made for the first 6 minutes, by which time the calorimeter and its contents had generally reached equilibrium and the temperature was changing at a constant rate   Thereafter the temperature was observed at intervals of exactly one minute

At the end of an experiment the cooling rate of the calorimeter was determined by removing the hand and closing the hole of its entry with a sorbo-rubber disc   Stirring was continued without interruption and the

temperature was recorded at one minute intervals for a period of ten minutes It would appear on theoretical grounds that the rate of cooling should be observed with the water in the calorimeter at several points on the temperature range used in the preceding experiment Alternatively, if the cooling rate is observed with the water at one temperature only, the cooling rate at other temperatures should be computed on the basis that it is proportionate to the difference between calorimeter and room temperatures Both methods have been employed in different experiments It soon became apparent, however, that the cooling rate of this type of calorimeter is dependent not only on the temperature of its contents at the time, but also, in a very large measure, on their temperature over a considerable period beforehand (10) This is no doubt due to complicated temperature gradients set up in the cork lagging and earthenware pot It is therefore impossible to make a really accurate cooling correction for the Stewart calorimeter The inaccuracy is undoubtedly much greater when it is used, as in these experiments, for following rates of heat elimination which change rapidly during the course of an experiment, than when it is used to measure a steady rate of heat elimination over a fairly long period The uncertainty of the cooling correction is most serious when the hand blood flow is so small that the rate of heat loss from the calorimeter considerably exceeds the rate of heat elimination from the hand When the blood flow through the hand is high, the heat loss by cooling is about one-sixth of the heat gain from the hand

A large number of experiments was performed with the Stewart calorimeter Because it is an instrument which has been much used, and because the uncertainties about the cooling connection do not invalidate the main features of the results, these experiments are reported now It was, however, evident that some comparable experiments ought to be performed with a calorimeter with more precisely definable physical properties Accordingly, the " vacuum " calorimeter (10) was employed , with this instrument, during control observations without the hand in place, the temperature of the water altered by less than 0 002°C per minute, or only one-fiftieth of the temperature change per minute produced by the hand when the vessels were widely dilated

At the end of all the calorimeter experiments the water level on the hand within the calorimeter was marked by introducing an oily dye on to the surface of the water and the volume of hand actually immersed was measured by displacement The results were calculated as calories/100 ml hand/min

Skin temperature Since the whole, or nearly the whole, hand was employed in the calorimeter or plethysmograph, it would have been appropriate to sample the skin temperature at many points on the surface of the hand, either by connecting in series a number of thermocouples disposed at various points, or by scanning in rotation many separate thermocouples However, as neither of these methods is in common use, observations were confined (unless otherwise noted) to the skin covering the

pulp of the middle finger   A single junction of gauge 32 copper wire and gauge 32 constantan wire was fixed in position by a single layer of adhesive tape   The latter was never made to encircle the terminal phalanx because this might have restricted the blood supply   The reference junction was in a thermos flask at 36°C, immersed in a large thermostatically controlled water-bath at the same temperature   The hand under observation was open and resting palm uppermost on the subject's lap

Production of changes in blood flow   The circulation through the hand was made to vary by heating or cooling other parts of the body (13)   As most of the experiments were performed in the summer, it was often impossible, by cooling the feet and leaving almost the whole body surface exposed, to reduce the hand blood flow to low levels, so that on hot days subjects spent a preliminary period of about 20 minutes in a cold room at 4°C, and then sat with their feet in a stirred water bath at 12 to 15°C until recordings started   In this way it was possible to commence an experiment with very low blood flows in the hands, but once the subject had been warmed it was often impossible to cool him again sufficiently to reduce the blood flows to the original level

The blood flow through the hands was increased by warming the subject with an electric blanket, placing both feet in a stirred water-bath at 42° to 44°C (9) and wrapping him in blankets   On some days fairly steady intermediate rates of blood flow were obtained by omitting active heating or cooling

RESULTS

The results fall into six groups   In each group experiments were performed at low, moderate and high levels of blood flow

Observations on parallel changes in blood flow through the two hands These observations were made to establish the degree of symmetry of the circulation through the hands   For this purpose the hands were both placed either in plethysmographs or in calorimeters, and conditions were maintained as nearly as possible identical on the two sides

(a) Calorimetric comparison   (5 experiments)

In these experiments an attempt was made to immerse equal volumes of hand in each calorimeter   As the two calorimeters were as nearly identical as possible and the experiments were commenced with the calorimeters at the same temperature, the uncertainty of the cooling corrections in no way detracts from the results   In each experiment the subject was warmed and cooled to alter his peripheral blood flow   Fig 1 shows the results obtained on D F, in whom the greatest range of heat elimination was observed   It illustrates the close minute to minute correspondence which was a feature of all the experiments   When the total amounts of heat eliminated from each hand during the whole experiment were compared (Table I) there was

likewise remarkably close agreement   This was so although the volumes of right and left hand immersed were not always identical   The volumes were widely different in the case of J G W S , the less immersed hand having the greater heat elimination per 100 ml tissue   The discrepancy in the two
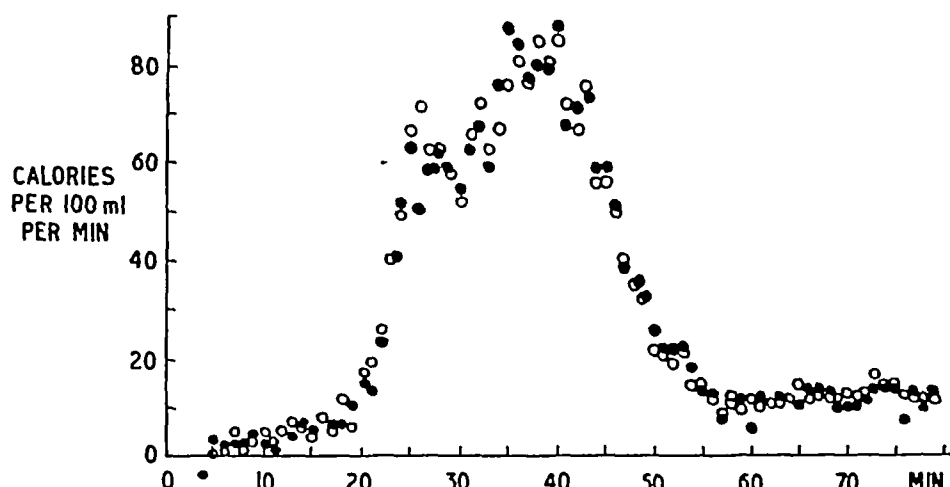

Fig 1   Comparison of the rates of heat elimination from the hands of D F into Stewart calorimeters
      Right hand—open circles   365 ml of hand immersed   Initial calorimeter temperature
      30 546°C , final temperature 31 842°C
      Left hand—solid circles   390 ml of hand immersed   Initial calorimeter temperature
      30 360°C , final temperature 31 705°C
Initial and final room temperature 22 3°C   Subject stripped and seated for 30 min before experiment started   At 18 min , blankets, hot pad, and stirred foot bath at 42 5°C started, and stopped at 47 min   At 55 min stirred foot bath at 15°C begun

hands may well be due to the difference in level of immersion   On a volume basis the heat elimination from the finger (5) is known to be greater than from the whole hand

TABLE I

Ratio of the total heat elimination from the two hands over a period during which the peripheral circulation was at various levels of constriction and dilatation.

Subject	Period of observation in minutes	Ratio of heat elimination per 100 ml of hand Right/Left	Volumes of hand immersed in ml	
			Right	Left
K E C	82	1 05	280	300
A D M G	85	0 97	320	320
H S	40	1 08	270	270
J G W S	67	0 84	520	400
D F	73	1 02	365	390

(b) *Plethysmographic comparison*   (3 experiments)

In this comparison it was easier to be sure at the outset that equal volumes of the two hands were enclosed in the instruments   The two plethysmographs were maintained at the same temperature throughout An example of these experiments is shown in Fig 2   Not only are the curves
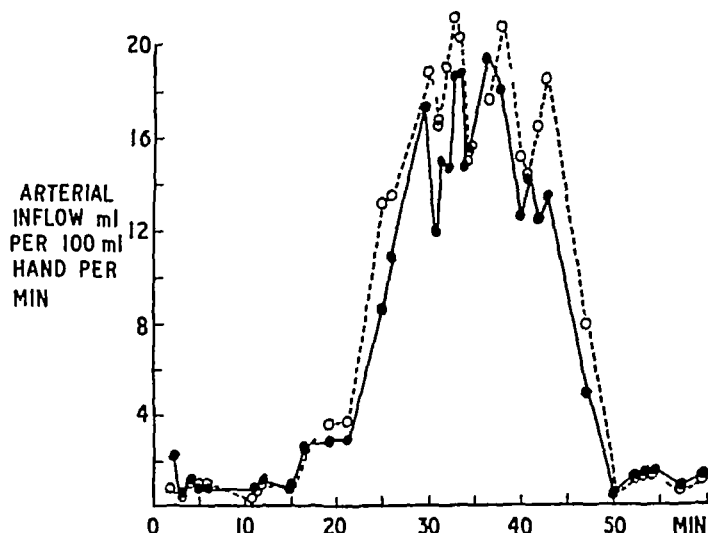

Fig 2   Comparison of the rates of blood flow through the hands of A P F when they were inserted into plethysmographs at the same temperature (30—30 5°C)   Volume of hand 460 ml on both sides

Right hand—open circles   Left hand—solid circles   Room temp 25°C   Subject stripped on seat for 30 min before experiment started   At 10 min blankets, hot pad and stirred foot bath at 42°C started, and stopped at 42 min

for the two hands very similar, but minute-to-minute fluctuations in the two hands occur simultaneously, as described by Freeman (8)   Table II shows that the total blood flows per 100 ml of hand throughout the experiments were approximately equal on the two sides

TABLE II

Ratio of the total blood flows through the two hands, per 100 ml of hand, over a period during which the flow varied between low and high values

Subject	Period of observation in minutes	Ratio of blood flows Right/Left	Volume of hand in plethysmograph in ml.	
			Right	Left
A.P.F	59	1 14	460	460
A.D.M G	62	1 10	460	460
D.F	65	0 92	420	420

Experiments to determine the influence of calorimeter temperature on the rate of heat elimination from the hand   These experiments were performed because it was found that in experiments comparing calorimetric with plethysmographic measurements over a period of an hour or more, the temperature in the calorimeter, usually 29 5-30 0°C at the start, had often reached 32-32 5°C at the end   The aim was to find out how much difference this temperature rise might make to the rate of heat elimination from the hand   Changes in temperature of the calorimeter water may mediate changes in heat elimination in two ways —

(*a*) By affecting the amount of heat released by each ml of blood flowing through the hand   This might be approximately allowed for on the basis that the heat release is proportional to the difference between body temperature and calorimeter temperature   Such a correction, however, does not allow for any redistribution of blood flow which might occur between superficial and deep vessels

(*b*) By altering the actual amount of blood flowing through the hand per minute (12, 8)

These experiments were made with Stewart calorimeters   On each of three subjects the heat eliminations from the two hands were measured simultaneously over 3 periods of about 20 minutes   Before every period the hands were equilibrated for 15 mins with the water that was to be used to fill the calorimeter   The aim was to have the water in the reference calorimeter about 30°C , and that in the other successively about 27, 30 and 33°C   Of the subjects used, one had a high, one a moderate and one a low heat elimination throughout the experiment   The results (Table III) show that under these conditions the rate of heat elimination from the hands followed fairly closely the difference between 37°C and the calorimeter temperature, over the range 27 to 33°C   Owing to the uncertainties which attend the making of any correction for different calorimeter temperatures and the complexities of the variables involved, it was not believed to be profitable to pursue this aspect of the problem further   It was decided, therefore, to adjust all results for heat elimination to the heat elimination that would be expected into a calorimeter at a temperature of 32°C , by using the formula —

$$\text{Adjusted rate of heat elimination} = \text{Observed rate of heat elimination} \times \frac{37°C - 32°C}{37°C - \text{Actual calorimeter temp}}$$

This adjustment is essentially similar to that used by Arnott and Macfie (1) for the finger calorimeter   All subsequent results described in this paper have been adjusted for calorimeter temperature in this way

TABLE III

Comparison of the rates of heat elimination from the two hands into calorimetres containing water at different temperatures   In each case the left hand was the reference hand   Each period of observation was 15 min   Heat eliminations are expressed in calories per 100 ml of hand per min

	PERIOD 1		PERIOD 2		PERIOD 3	
Subject D F	Right	Left	Right	Left	Right	Left
Average calorimeter temp °C	26 9	30 2	30 7	31 3	32 8	31 1
Average heat elimination	17 2	14 3	13 4	13 0	11 5	13 4
Ratio of heat eliminations R/L	1 23		1 03		0 86	
$\dfrac{37°C - R \text{ calorimeter temp °C}}{37°C - L \text{ calorimeter temp °C}}$	1 48		1 10		0 71	
Subject K.E C						
Average calorimeter temp °C	28 6	31 2	30 15	30 76	32 7	31 2
Average heat elimination	161	104	120	112	73	113
Ratio of heat eliminations R/L	1 55		1 07		0 62	
$\dfrac{37°C - R \text{ calorimeter temp °C}}{37°C - L \text{ calorimeter temp °C}}$	1 45		1 09		0 74	
Subject A.D.M G						
Average calorimeter temp °C	27 2	30 6	30 5	30 8	32 7	31 2
Average heat elimination	74	49	53	46	32	62
Ratio of heat eliminations R/L	1 51		1 15		0 51	
$\dfrac{37°C - R \text{ calorimeter temp °C}}{37°C - L \text{ calorimeter temp °C}}$	1 53		1 05		0 50	

Experiments to determine the effect of plethysmograph temperature on the rate of blood flow through the hand   Freeman (8) has investigated the effect of local temperature on the blood flow through the hand   His subjects had blood flows of from 2 to 6 ml per 100 ml of hand per minute at a plethysmograph temperature of 30°C   We have extended these observations to subjects with the extremities in a state of general vasoconstriction or general vasodilatation produced by the cooling or heating   In ten experiments the temperature of the water in the plethysmograph was altered on two or three occasions during a single experiment and maintained at the new temperature for 15-20 min   The heat elimination from the other (reference) hand was meanwhile observed in a calorimeter at 30-32°C   It was fully realised that the periods of observation were shorter than desirable, but they could not

be increased without making the experiments so long that they were uncomfortable for the subject, and required changes of water in the calorimeter to keep its temperature within the range 29 5 to 32°C   Change of calorimeter water was avoided if possible and when it became necessary no reliance was placed on results obtained in the first 15 minutes after the change, owing to considerations mentioned when we discussed the properties of the Stewart calorimeter   The blood flow appeared to settle at a new level within a few minutes of changing the plethysmograph temperature   Results obtained before it had done so have been discarded

An attempt was made to test different subjects at different levels of blood flow through the hands, the circulation being maintained in as stable a state as possible during each experiment   When the peripheral vascular bed is well dilated, or well constricted, reasonably constant levels of blood flow can be maintained for 30 minutes or more, but it is difficult to achieve this when the circulation is not in one of the extreme conditions   Observations of the heat elimination from the reference hand permitted some allowance to be made for uncontrollable alterations in the circulation as a whole, as opposed to the local effect of the plethysmograph temperature   At each plethysmograph temperature range in each experiment the mean blood flow and heat elimination were determined from readings at minute intervals over at least 10 min   At plethysmograph temperatures other than 30°C the blood flow figures have been adjusted for alterations in the general state of the circulation during an experiment by use of the expression —

$$\text{Average blood flow observed} \times \frac{\text{Average heat elimination from reference hand per minute while plethysmograph was at } 30°\text{ C}}{\text{Average heat elimination from reference hand per minute while blood flow was being observed}}$$

The temperature 30°C was chosen because, following Grant and Pearson (6) many workers have maintained the plethysmograph at this temperature It is also within the temperature range used in the calorimeter   This allowance is not entirely satisfactory because it assumes that any alteration in the general state of the circulation during an experiment would cause the blood flow through the two hands (under different local conditions) to be altered in the same proportion   It is also ultimately dependent on the corrections applied to the Stewart calorimeter when it is being used at temperatures other than 32°C   It is nevertheless thought to be reasonably satisfactory

The results of these experiments are shown in Fig 3   The blood flow through the hand is appreciably altered by local temperature changes over a range which includes temperatures normally employed in the calorimeter

Such alterations are somewhat greater at low flows than at high ones   Our results for subjects in an intermediate state of peripheral vasodilatation are similar to those of Freeman (8), but we recorded higher flows at plethysmograph temperatures of 40-43°C
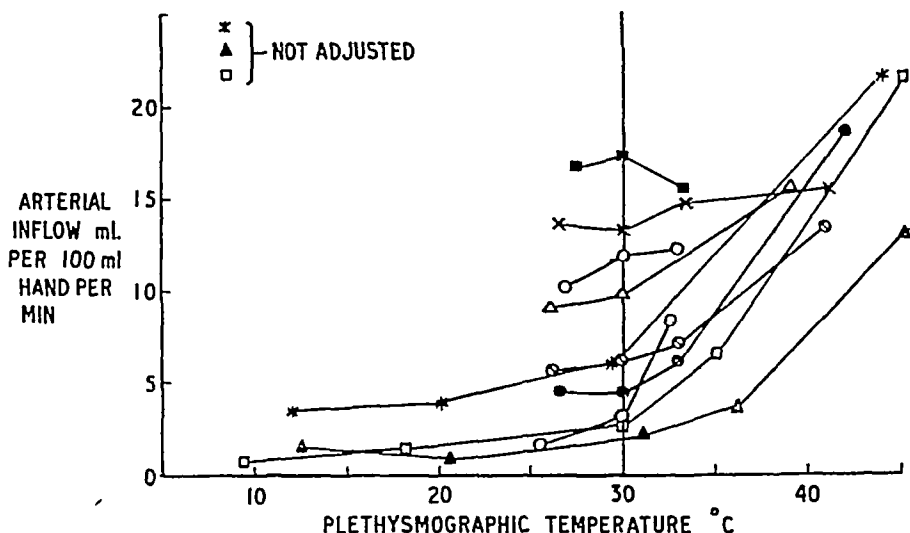

Fig 3   The effect of local temperature on the blood flow through the hand as measured by the plethysmograph   Flows at temperatures other than 30°C have been adjusted for changes in the general state of the circulation as described in the text   This adjustment was omitted in the three experiments indicated, because the heat elimination from the reference hand was too small to be measured with accuracy   Each point represents the average of blood flows observed at minute intervals for not less than 10 min

Experiments in which the heat elimination from one hand was compared with the blood flow, as measured by the plethysmograph, through the other hand In a series of five experiments during which the plethysmograph temperature throughout the experiment was kept within 0 2°C of the temperature of the Stewart calorimeter, the blood flow through the hands was maintained in a relatively steady state for periods of at least 10 min   Successive periods in each experiment covered a wide range of flow, from about 1 to 16 ml per 100 ml of hand per min   The relationship between the mean heat elimination per min and the mean blood flow per min over a number of such 10 min periods is shown in Fig 4

In these experiments the hands were strictly comparable as regards temperature   They differed only in position, and in the volume under observation   The average volume in the plethysmograph was about 400 ml , and in the calorimeter about 300 ml   At low flows about 3 8 calories are

eliminated from each ml of blood, but at high flows some observations show a heat elimination of between 5 5 and 6 calories per ml of blood   Possible explanations of this discrepancy are —

1   That the fingers form a greater proportion of the smaller volume of hand in the calorimeter than of the larger volume of hand in the plethysmograph   It is known that the fingers normally carry a greater blood flow in relation to their volume when the vessels are dilated than does the hand (5, 17)

2   That the plethysmographic observations are inaccurate at very high rates of flow   It often happens then that the hand vessels become filled during the three or four heart beats immediately following the application of the collecting pressure, and measurements may depend on a tangent drawn to only two or three pulse waves
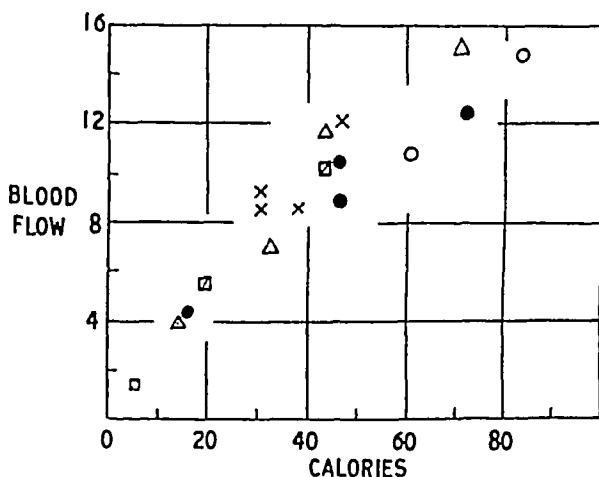
Fig 4   Comparison of the rate of heat elimination from one hand into a Stewart calorimeter with the rate of blood flow through the other hand as measured with a plethysmograph The water in the plethysmograph was maintained at the same temperature as that in the calorimeter   Heat elimination in calories per 100 ml of hand per min , adjusted to calorimeter temperature of 32°C   Blood flow in ml per 100 ml of hand per min.   Each point represents the average of observations over a period of not less than 10 min

To exclude the first of these possible sources of error, a second series of four experiments was performed in which equal volumes of hand were employed in the plethysmograph and calorimeter   In this series the "vacuum" calorimeter was used, and the plethysmograph temperature was maintained within 0 1°C of that in the calorimeter   On account of the large and rapid fluctuations which are known to occur, particularly at rates of flow intermediate between vaso-constriction and vaso-dilatation, plethysmographic observations were made two or three times in every minute

The results of a typical experiment are shown in Fig 5   During the first 45 min the rate of heat elimination was intermediate between that consistent with full vasoconstriction and full vasodilatation   Throughout

this period the plethysmograph showed rapid fluctuations of flow in the other hand, successive readings varying between zero and 18 ml per 100 ml per min (Fig 5A) Since it has been shown that such variations of flow occur simultaneously in the two hands it is evident that the calorimeter gives a more useful picture of the average state of the circulation over a
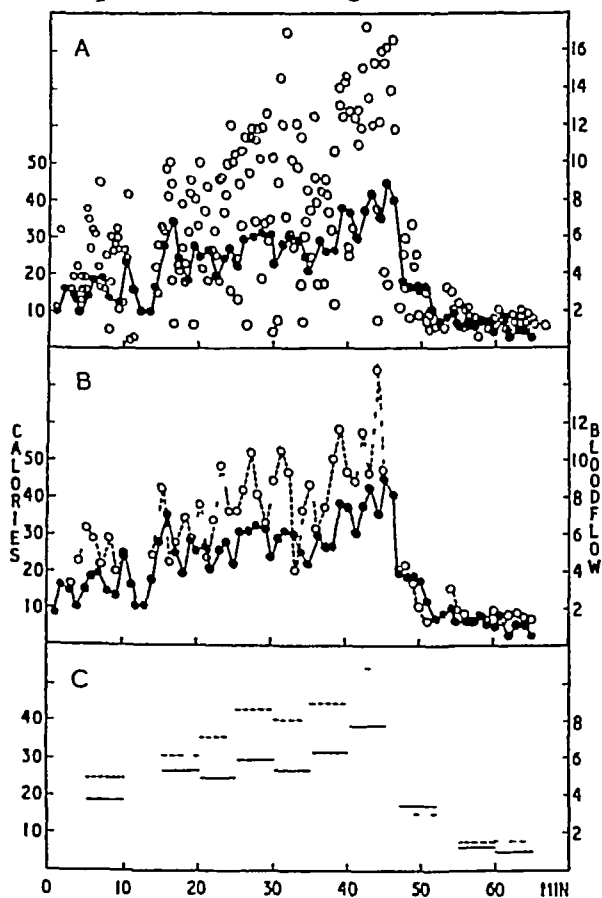

Fig 5   Simultaneous observations of heat elimination from the right hand into a vacuum calorimeter, and volume flow through the left hand as measured with a plethysmograph at the same temperature as the calorimeter   Subject, K W C
     Open circles and broken lines, blood flows in ml per 100 ml of hand per min   Solid circles and continuous lines, heat elimination in calories per 100 ml of hand per min adjusted to calorimeter temperature 32°C   Volume of right hand immersed, 500 ml , volume of left hand in plethysmograph, 490 ml
A   Actual observations, plethysmographic determinations two or three times per min
B   Blood flows averaged for each min
C   Blood flows and heat eliminations averaged over 5 min periods

period than does the plethysmograph   These violent fluctuations of flow, which the calorimeter is incapable of recording, are much less evident both when the vasomotor tone is greatly increased (Fig 5A from 50th min),

and when the vessels are widely dilated　　When the volume flows measured with the plethysmograph during each minute are averaged, then the fluctuations of these averages are less striking, and the correspondence between them and the heat eliminations per minute is less irregular (Fig 5B) The parallelism is much closer when the results are expressed as the mean of the readings during five minute periods (Fig 5C)　　When this is done, there is at all levels of vasodilatation a heat elimination of about 4 cals per ml blood flow (Fig 6)
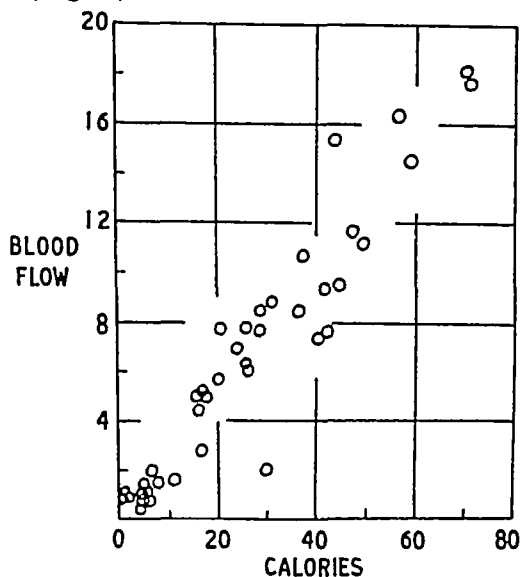
Fig 6　Comparison of the rate of heat elimination from one hand into a vacuum calorimeter with the rate of blood flow through the other hand as measured with a plethysmograph Volumes of hand observed, and local temperatures as nearly as possible equal
Blood flow in ml per 100 ml of hand per min　　Heat elimination in calories per 100 ml of hand per min　adjusted to calorimeter temperature of 32°C
Each point represents the average of observations over a period of not less than 10 min

In general, during periods of circulatory stability there was remarkable agreement between the plethysmograph and the calorimeter, a finding which increased our confidence in the general reliability of both methods

Comparison between the heat elimination from one hand, and the skin temperature measured over the pulp of the middle finger of the other hand　　In each of the eight experiments in which this comparison was made, vasoconstriction and vasodilatation to varying degrees were maintained by immersing the feet in cold or hot water , the room temperature was between 20 and 24°C　　Results from two typical experiments are shown in Fig 7　Fig 7A shows an experiment on K E C in which striking changes in skin temperature over the range 22—34°C accompany quite small alterations in heat elimination　　Fig 7B shows another experiment on the same subject in which large and rapid alterations in the heat elimination from the hand were produced by rapid heating and cooling of the legs and trunk　It can be

seen that the rise in skin temperature kept pace with the rapid rise in heat elimination, but the skin temperature lagged behind when the heat elimination fell abruptly   The skin temperature decline in the latter case was very similar to that seen when the circulation to the arm is arrested   The rate of decline then depends on the rate at which the hand loses heat to its surroundings   The calorimeter responds quickly to a decrease or arrest of blood flow with a drop in heat elimination, which represents simply a failure of the system to gain heat
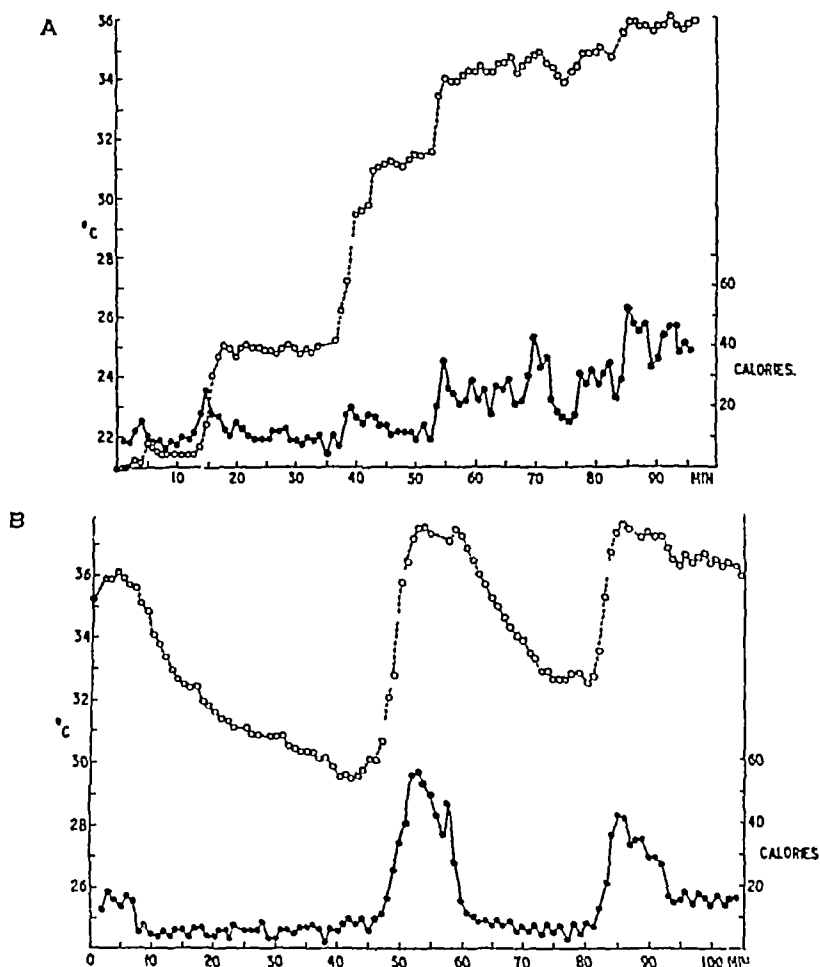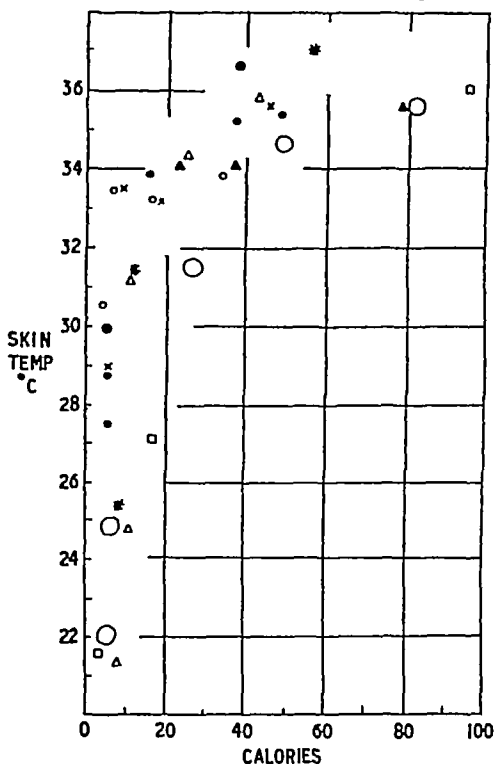

Fig 7   Comparison of the heat elimination from the left hand of K E C with the skin temperature measured over the pulp of the terminal phalanx of the middle finger of the right hand Open circles and broken lines, skin temperature in °C   Solid circles and continuous lines, heat elimination in calories per 100 ml of hand per min adjusted to calorimeter temperature of 32°C
A   Subject cold at start, and warmed in stages   Room temp 20 8 21 3°C
B   Subject alternately warmed and cooled   Room temp 21 7 22 0°C

The means of the skin temperature and heat elimination readings over periods of several minutes when both were reasonably stable are shown plotted against each other in Fig 8, from which it is clear that skin temperatures of 34°C were obtained when the heat elimination from the other hand was a quarter or less of the maximum obtainable by vasodilatation in response to heating   Above 34°C the skin temperature increased only slightly with large increases in heat elimination from the other hand

The heat elimination from one fifth finger (14) was measured in a few experiments simultaneously with the skin temperature of the other fifth finger   The results were very similar to those already quoted for hand calorimetry, and are not separately reported

Since the surface temperature of the hand in the calorimeter was at all times within the range 30-32°C , while that of the other hand exposed to the air varied from 16 to 36 5°C , it may be supposed that at any particular time there were considerable differences in blood flow between the two hands It is reasonable to expect that when the skin temperature was below 30°C ,

Fig 8   Comparison of the rate of heat elimination from one hand with the skin temperature as measured over the pulp of the terminal phalanx of the middle finger of the other hand Results from 8 experiments at a room temperature of 20 24°C , and one (large circles) perform ed in February at a room temperature of 12°C   Heat elimination in calories per 100 ml of hand per min , adjusted to calorimeter temperature of 32°C   Each point represents the average of observations over a period of about 10 min during which the conditions were reasonably stable

the blood flow in that hand was less than in the calorimeter hand, that between 30°C and 32°C it was the same, and that above 32°C it was greater If such is the case, for Fig 8 to represent the relationship of skin temperature to the blood flow in the same hand, the points below 30°C would have to be shifted to the left, and those above 32°C to the right   Whether or not this is done, it is clear that at low blood flows, small variations in flow cause big changes in skin temperature, but that at high blood flows, big variations in flow cause small changes in skin temperature   These results are similar to those obtained by Wright and Phelps (18) in a comparison of skin temperature with blood flow in the foot and leg

A further comparison on three subjects was made during cold weather in the following February   The results of one of these experiments, made at a room temperature of 12°C   are included in Fig 8   The points lie rather to the right of the previous series, but the essential features of the curves on which they lie are similar

SUMMARY AND CONCLUSIONS

(1)   Experiments have been carried out on eight healthy male subjects in which the blood flow through the hand was assessed by three methods, namely, the venous occlusion plethysmograph, (arterial inflow), the hand calorimeter (heat elimination), and surface thermocouple (skin temperature) The methods were compared by using them simultaneously in pairs on the two hands

(2)   When compared over periods of time the arterial inflow was remarkably similar in the two hands and so was the heat elimination   This was so at all levels of peripheral vasodilatation and vasoconstriction Minute-to-minute fluctuations in both volume flow and heat elimination were symmetrical in the two hands

(3)   The effect upon arterial inflow of varying the plethysmograph temperature has been re-investigated taking into account, and allowing for, alterations in the general circulation during the period of the observations When the arterial inflow was rapid as a result of heating part of the body the effects of local temperature changes were relatively small and unimportant, whereas at low and intermediate rates of inflow the effect of changes of plethysmograph temperature were more important

(4)   During periods of some minutes, when the circulation through the hands was reasonably stable, the rate of heat elimination from one hand closely paralleled the average rate of arterial inflow into the other hand, both hands being at the same temperature

(5)   Under these conditions, the heat elimination was about 4 calories per 1 ml of blood, at all levels of vasodilatation

(6) At intermediate levels of vasodilatation the arterial inflow may vary greatly in the course of a single minute, and a true picture of the general level of blood flow can only be obtained by averaging a large number of observations   The slower response of the calorimeter smooths out these rapid fluctuations

(7) The response of the calorimeter is sufficiently fast to follow with considerable accuracy a large increase or decrease in the blood flow occurring over a period of two or three minutes

(8) In a subject who is cool at the beginning of the experiment with a skin temperature at or below room temperature (20-24°C), a skin temperature of 34°C is usually attained on the pulp of the middle finger of one hand when the heat elimination from the other hand is a quarter or less of the maximum obtainable by heating the subject   When the skin temperature has reached 34°C considerable increases in heat elimination are accompanied by small increases in skin temperature, up to a maximum of 36 5°-37°C

(9) Skin temperature responds promptly to increases in the blood flow occurring over periods of two to three minutes, but if the blood flow falls abruptly, the skin temperature falls slowly, as it does when the circulation is arrested

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THE MECHANISM OF THE REDUCTION OF CLOT RETRACTION BY SEDORMID IN THE BLOOD OF PATIENTS WHO HAVE RECOVERED FROM SEDORMID PURPURA

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IT has been shown (2) that when sedormid is added to the blood of patients who have recovered from sedormid purpura, clot retraction is reduced, and in some patients the platelets are agglutinated   These effects are not seen in the blood of normal individuals   It is well known that if platelets are removed from plasma by centrifugation (3, 5, 6, 7) or by the action of an anti-platelet serum (7), then clot retraction is reduced or even abolished   It therefore seemed probable that the action of sedormid in reducing clot retraction might be due to an effect on the platelets   As, however, agglutination of platelets could not be demonstrated in the blood of all the patients in whom sedormid caused a reduction in clot retraction and as lysis of platelets by sedormid was not observed in any of these preparations, it seemed possible that the reduction in clot retraction might be due to an effect of sedormid on some other part of the clotting mechanism   Fibrinogen, thrombin and platelets were therefore added to different samples of the blood of patients who had recovered from sedormid purpura and the action of sedormid on clot retraction was again investigated on the supposition that a normal clotting factor might replace one sensitive to sedormid and so permit normal clot retraction in the presence of the drug   It is the purpose of this paper to record the results of these experiments, and those to which they led, and to show that, although, under the conditions of the experiments previously reported, sedormid does not cause lysis of platelets in citrated blood, yet an abnormally rapid lysis of platelets does occur when the blood of these patients is allowed to clot in the presence of the drug   It is also shown that this lysis of platelets is due to some factor in the plasma, and is not due to a direct interaction of sedormid with the platelets of sensitised individuals

The cases investigated (Case I (N H), Case II (M B) and Case III (J MacP)) are those described in a previous communication (2)

Methods

Preparation of solutions of sedormid   Saturated solutions of sedormid in 3 2% sodium citrate, 0 9% sodium chloride and in various preparations of

"citrate saline" (isotonic mixtures of these two solutions) were prepared by boiling these preparations for a few seconds with the pure chemical allyl-isopropyl-acetyl-carbamide, which is the active principle of sedormid, allowing them to stand at room temperature over night and then filtering   For the sake of brevity, such solutions are referred to throughout this paper as solutions of sedormid

    Estimation of clot retraction   This was done by a method previously described (1)

    Platelet counts   These were done on plasma after suitable dilution with 3 2% sodium citrate   The diluted plasma was mixed for five minutes in a mechanical shaker before being transferred to the haemocytometer

Effect of the addition of normal fibrinogen to whole blood on the inhibition of clot retraction by sedormid

The fibrinogen was kindly supplied by the Medical Research Council Blood Products Unit, to which I am indebted for generous supplies both of human fibrinogen and human thrombin   This fibrinogen is made up in citrate saline and lyophilised   When dissolved in water to produce a concentration of 1 0% fibrinogen, the fluid has a salt content of 0 88% sodium chloride and 0 37% sodium citrate   The sodium citrate was neutralised by the addition of 2 0 ml of isotonic (1 18%) calcium chloride to 10 0 ml of fibrinogen solution   0 63 ml of this recalcified fibrinogen solution was added to 2 0 ml of blood to produce a final concentration of

TABLE I

Effect on clot retraction of the addition of redissolved lyophilised human fibrinogen to blood showing no abnormality of coagulation

Patient	Diagnosis	Concentration of added fibrinogen	No of determinations for each experiment	Clot retraction % without added fibrinogen	Clot retraction % with added fibrinogen
H A	Chronic cholecystitis	0 2%	4	59	18
A V	Rheumatic heart disease	0 2%	4	62	22
M O	Coronary thrombosis	0 2%	4	59	34
S W	Constipation	0 1%	4	59	49
B U	Pleural effusion (probably tuberculous)	0 1%	4	60	50
H E	Healed lobar pneumonia	0 1%	4	58	41

0 2% of added fibrinogen   The retraction of clots formed from this mixture was compared with that of 2 0 ml of blood diluted with 0 63 ml of recalcified citrate saline alone   It was found that the fibrinogen caused a marked reduction in clot retraction   This effect was still seen if the concentration of added fibrinogen was reduced to 0 1% although the reduction in clot retraction was then much less   These results were obtained both with samples of normal blood (Table I) and with blood from patients who had recovered from sedormid purpura (Table II)   This effect of fibrinogen is surprising but it receives confirmation from some experiments of Lundsteen (5) who showed that if the concentration of fibrinogen in plasma was lowered then clot retraction (measured by the rate of expression of serum) was increased

TABLE II

Effect on the inhibition of clot retraction by sedormid of the addition of redissolved lyophilised fibrinogen to the blood of two patients who had recovered from sedormid purpura

Patient	Concentration of added fibrinogen	No of determinations for each experiment	Clot retraction % without added fibrinogen		Clot retraction % with added fibrinogen	
			Without sedormid	With sedormid	Without sedormid	With sedormid
Case I (N H)	0 2%	4	71	20	32	6
	0 1%	4	72	34	55	10
Case II (M B)	0 1%	4	66	41	48	20

The effect of the addition of sedormid and fibrinogen to the blood of patients who had recovered from sedormid purpura was subsequently investigated by using the same solutions saturated with sedormid   It was found that when these two substances were added to the blood of these patients, clot retraction was almost entirely abolished and, moreover, that the fibrinogen and sedormid each appeared to augment the reduction in clot retraction due to the other   These findings are summarised in Table II

If the reduction in clot retraction observed when sedormid was added to the blood of patients who had recovered from sedormid purpura was due to an action of sedormid on their fibrinogen, then the addition of normal nonsensitive fibrinogen would have been expected to improve clot retraction in the presence of the drug, probably to the level produced by the addition of fibrinogen alone, and certainly not to reduce it to a level even lower than that produced when sedormid was added by itself   It therefore seems improbable that there was any replacement of sensitive by non-sensitive fibrinogen in these experiments, unless there was also some other factor involved in the inhibition of clot retraction by sedormid

Effect of the addition of normal thrombin to whole blood on the inhibition of clot retraction by sedormid

When thrombin is added to normal blood, clot retraction is generally slightly reduced (*see* Table III)   This is because clots produced by thrombin tend to be more bulky than normal as fewer cells are extravasated into the serum (1)   This same effect was observed when thrombin was added to

TABLE III

The effect on clot retraction of the addition of thrombin to blood of patients with no abnormalities of coagulation

Patient	Diagnosis	2 0 ml blood + 0 1 ml saline		2 0 ml blood + 5 units thrombin in 0 1 ml saline	
		No of experiments	Clot retraction %	No of experiments	Clot retraction %
T D	Silicosis	2	56	4	52
N O	Healed coronary thrombosis	3	50	4	51
W A	Healthy man	4	56	4	55
B L	Healthy man	4	54	4	48
M I	Healthy man	4	50	4	49
J A	Healthy man	4	54	4	50

the blood of two patients who had recovered from sedormid purpura   It was found, however, that if blood from these patients was added to a saturated solution of sedormid in saline to which thrombin had previously been added, the reduction in clot retraction was less than that produced by a saturated solution of sedormid in saline alone (*see* Table IV)   It seemed possible that this result might be due to the replacement with normal thrombin of thrombin modified in some way by sedormid, or alternatively, that the thrombin so greatly accelerated clotting that sedormid had not time to act as completely as usual   To investigate this latter point, coagulation was delayed by the use of sodium citrate, and the action of thrombin on the inhibition of clot retraction by sedormid was investigated again to see if delaying clotting for several minutes before the thrombin was added would enable the sedormid to exert its usual effect on clot retraction by allowing the drug a longer period in which to act   Samples of blood were added to a saturated solution of sedormid in citrate saline in clot retraction tubes which also contained thrombin   Further samples were citrated with the same sedormid containing solution and were clotted by adding thrombin after a

TABLE IV

Effect of thrombin on the inhibition of clot retraction by sedormid in the blood of patients who have recovered from sedormid purpura

Details of experiment	Case I* (N.H.)		Case II (M B)	
	No of experiments	Clot retraction %	No of experiments	Clot retraction %
0 5 ml saline + 2 0 ml. blood	5	69	4	67
0 5 ml saline + 0 1 ml saline containing 4 units of thrombin + 2 0 ml blood	5	68	4	60
0 5 ml saturated solution of sedormid in saline + 2 0 ml blood	5	15	4	12
0 5 ml saturated solution of sedormid in saline + 0 1 ml saline containing 4 units of thrombin + 2 0 ml blood	5	29	4	31

period of 5 minutes   The clot retraction of all these preparations was then estimated and compared with that of citrated blood clotted with thrombin alone   The results of these experiments are summarised in Table V   They show that when clotting was not delayed, thrombin was able to reduce the action of sedormid quite considerably but that the action of sedormid in reducing clot retraction was preserved if the thrombin was not added for about five minutes   It therefore seemed clear that the effect of thrombin in reducing the action of sedormid on clot retraction was due solely to its power to accelerate coagulation

Lysis of platelets by sedormid during coagulation

The above experiments show that sedormid requires a certain time in which to act, and that only if the blood is clotted after this period has elapsed, will the full effect of sedormid on clot retraction be obtained   This observation raised the problem as to what is happening to the blood during this period   As these experiments had failed to provide any evidence that sedormid reduces clot retraction by an action on fibrinogen or thrombin it seemed possible that this period might represent the time taken by sedormid to produce some alteration in the platelets   The platelets were therefore observed during coagulation in the following ways

1   *By making Leishman stained films of clotting blood*   Blood was taken from an arm vein in an oiled syringe and 2 0 ml was added to 0 5 ml of a saturated solution of sedormid in saline in a test tube   A further 2 0 ml

* Bovine thrombin used in these experiments

TABLE V

Effect of thrombin on the action of sedormid on the clot retraction of citrated blood from sedormid sensitive patients

Patient	Details of experiment	Coagulant	No of determinations for each experiment	Clot retraction % without sedormid	Clot retraction % Coagulant added to a saturated solution of sedormid in citrate saline before blood added	Clot retraction % Coagulant added 5 mins after blood added to a saturated solution of sedormid in citrate saline
Case I (N.H)	2 0 ml blood + 0 5 ml citrate saline (0 37% sodium citrate in 0 88% sodium chloride)	4 units thrombin in 0 2 ml 1 18% calcium chloride	4	62	50	13
	Ditto	Ditto	3	62	51	20
	2 0 ml blood + 0 5 ml citrate saline (0 8% sodium citrate in 0 7% sodium chloride)	4 units thrombin in 0 3 ml 1 18% calcium chloride	4	63	53	28
Case II (M B)	2 0 ml. blood + 0 5 ml citrate saline (0 37% sodium citrate in 0 88% sodium chloride)	4 units thrombin in 0 1 ml saline	4	62	53	10

of blood, diluted with 0 5 ml of saline alone was used as a control Each preparation was mixed by inversion Samples, from which dry films were made, were aspirated every minute from each tube until clotting was so far advanced that samples could no longer be readily obtained The films were later stained with Leishman's stain In Cases I and II, the platelets in both preparations underwent agglutination but this tended to occur earlier in the sedormid than in the saline preparations The platelets in saline subsequently underwent only quite slow lysis, whereas in the sedormid preparations the agglutinated platelets underwent rapid lysis This was most strikingly demonstrated in preparations from Case II who was the most sensitive patient examined The earliest change occurred in the periphery of the agglutinates where the platelets often lost their definition and staining characteristics as early as two minutes after the blood was added to the sedormid solution At about the same time, the platelets in the centre of the agglutinates often became fused into a structureless mass Lysis proceeded rapidly and the platelets had usually been reduced to a very few barely recognisable, faintly staining objects before clotting began The appearance of the agglutinated platelets undergoing lysis is well shown in Fig 1 Similar but less striking changes were observed in the blood of Case I (N H) but on one occasion no lysis was seen It seems probable, from the observations recorded in the next paragraph, that the platelets did not undergo lysis in this preparation until after clotting had occurred and was sufficiently advanced to prevent the aspiration of specimens In Case III (J MacP), who was a much less sensitive patient, no abnormal lysis was observed The findings in all these cases are summarised in Table VI

2 *By observing coagulation of platelet rich plasma in a hæmocytometer chamber* Blood was taken in an oiled syringe and 10 0 ml transferred to each of two waxed centrifuge tubes These were immediately centrifuged at 2,500 r p m for 90 seconds This caused sedimentation of most of the red and white cells leaving a layer of platelet rich plasma above With a waxed pipette, 40 drops of this platelet rich plasma were transferred to each of two more waxed tubes, one containing 10 drops of saline and the other, 10 drops of a saturated solution of sedormid in saline The two tubes were closed with waxed corks and mixed by inversion A drop from each was transferred to a hæmocytometer chamber and the two preparations were observed under the microscope Sometimes the blood samples were added to the sedormid and saline in the proportion of 1 0 ml of saline or sedormid solution in saline to 4 0 ml of blood and then centrifuged The supernatant platelet rich plasma was immediately transferred to the hæmocytometer chamber This was found to be a satisfactory method as long as centrifugation was complete before the sedormid had caused any significant degree of platelet agglutination If, however, agglutination occurred before the blood had been centrifuged, then the agglutinated platelets were thrown

TABLE VI

Agglutination and lysis of platelets by sedormid during coagulation of blood of sedormid sensitive patients

Case	Date 1946	Coagulation time.* Sedormid	Saline	Appearance of platelets in stained films † — Platelet agglutination.* Sedormid	Saline	Platelet lysis in sedormid preparation.*	Date 1946	Expt.‡	Coagulation time.* Sedormid	Saline	Appearance of platelets in haemocytometer chamber.* — Platelet agglutination.* Sedormid	Saline	Platelet lysis in sedormid preparations.*
I (N.H.)	4-6	4 mins.	10 mins.	1½ mins.	>10 mins.	No abnormal lysis observed	4-6	B	—	—	—	—	Lysis observed only after clotting occurred
	8-10	—	—	—	—	Lysis definite at 7 mins.		B	Coagulation delayed by keeping plasma + saline or sedormid in waxed tubes before transferring to haemocytometer		—	—	Lysis observed before clotting occurred
	29-10	6½ mins.	8½ mins.	1 min.	2 mins.	Lysis definite at 1½ mins	29-10	A	12 mins.	15 mins.	7 mins.	>11 mins	Lysis definite at 7 mins. Almost complete at 9 mins.
II (M.B.)	30-4	—	—	—	—	Lysis definite at 1 mins. Almost complete at 6 mins.	10-10	B	6 mins.	9 mins.	1½ mins.	5 mins.	Lysis definite at 4½ mins. Almost complete at 7½ mins
	28-9	—	—	1 min	1 min	Lysis definite at ½ mins Complete at 4 mins.	25-10	A	10½ mins.	17½ mins.	4 mins.	>10 mins.	Lysis definite at 5 mins. Almost complete at 8 mins.
	9-10	—	—	—	—	Lysis definite at ½ mins Complete at 5 mins							
III (J MacP)	14-10	7 mins.	9 mins.	Agglutination only slight	Agglutination only slight	No abnormal lysis observed.	11-11	A	9 mins.	11 mins.	Agglutination only slight	Agglutination only slight	No abnormal lysis observed
	16-10	7½ mins.	>12½ mins.	ditto	ditto	ditto							
	9-11	9 mins.	9 mins.	ditto	ditto	ditto							

* The times given are measured from the moment the blood or plasma added to saline or sedormid in saline. The term lysis refers to the morphological changes in the platelets which are due to sedormid, and which are described in the text. No attempt was made to count the platelets or to measure the degree of agglutination and the rate at which the platelets disintegrate during coagulation made this impossible.

† 2·0 ml. blood mixed with 0·5 ml saline or sedormid in saline. Samples were aspirated at minute intervals and dry films were made which were later stained with Leishman's stain

‡ Expt. A. Platelet rich plasma prepared by centrifuging 4·0 ml. blood mixed with 1·0 ml saline or sedormid in saline.
Expt. B. Platelet rich plasma prepared by centrifuging whole blood without the use of anticoagulant. 4 volumes of plasma then mixed with 1 volume of saline or

down during centrifugation and the supernatant plasma became practically free of platelets   All three patients and a series of controls were investigated by one or other of these methods   In Cases I and II a very similar picture was observed   Agglutination of platelets occurred earlier and was much more marked in sedormid than in saline   The platelets in the saline preparations remained visible as highly refractile bodies until about the time clotting began and could be seen distinctly for a considerable period after this, whereas the platelets in the sedormid preparations rapidly lost this characteristic refractility and gradually became less and less distinct until finally all that remained were a few ill-defined amorphous masses   It was often difficult to say when lysis was complete   In most cases this process of platelet lysis was well advanced before clotting began but on one occasion, in Case I (N H), the coagulation time in both the saline and the sedormid preparations was unusually short and lysis of platelets did not occur until after the plasma had clotted   The lysis of platelets on this occasion was, however, just as definite, the fibrin threads enmeshing large numbers of platelets in the saline preparation, while only an occasional platelet could be seen in the sedormid preparation   A few minutes after this experiment was completed a further sample of blood was taken and treated in the same way except that the specimens of platelet rich plasma, which had been mixed with saline or sedormid, were left in the waxed tubes for five minutes before being transferred to the hæmocytometer   On this occasion the lysis of platelets by sedormid occurred before clotting began   No abnormal lysis of platelets by sedormid was observed in the plasma of Case III (J MacP) or in plasma from twelve controls   The findings in the three patients who had recovered from sedormid purpura are summarised in Table VI   The coagulation times of many of the samples are also shown in this table   It will be seen that with only one exception, in which the clotting times were equal, the samples containing sedormid clotted considerably earlier than did the samples which contained saline alone   It seems probable that this acceleration of coagulation was due to the liberation of thrombokinase and possibly also of thrombin (4), by the platelets which were undergoing lysis The coagulation times of the saline and sedormid preparations were accurately measured in six of the twelve controls   On no occasion did they differ by more than 30 seconds   A photomicrograph showing the lysis of platelets by sedormid in a hæmocytometer chamber during the clotting of plasma from Case II (M B) is shown in Fig 2

It appears from these experiments that sedormid causes lysis of platelets during the coagulation of the blood of some patients who have recovered from sedormid purpura   This lysis begins from 2-7 minutes, and is almost complete from 4-9 minutes after exposure of the blood to sedormid Tocantins (7) has produced a considerable amount of evidence to show that platelets cause clot retraction by settling on the fibrin threads and later fusing into small masses, so drawing the fibrin strands together   It is clear

that if this explanation is correct then the lysis of platelets by sedormid during coagulation explains the loss of clot retraction which has been observed when sedormid is added to the blood of patients who have recovered from purpura due to this drug   Since the lysis of platelets by sedormid may not occur until after clotting has started, it would seem that the explanation of the antagonistic action of thrombin noted above cannot be merely that it precipitates clotting before the abnormal platelet lysis due to sedormid has occurred
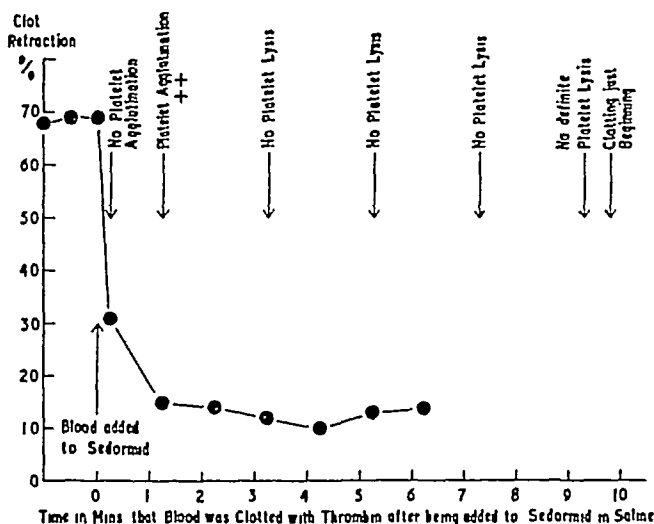
Fig 3   Graph showing the effect of thrombin on the inhibition of clot retraction by a saturated solution of sedormid in saline and the action of sedormid on the platelets in a similar preparation made at the same time   Blood taken from Case I (N.H)   As in Table VI, the term lysis refers only to morphological changes in the platelets

To investigate further the action of thrombin, 40 ml of blood were transferred from an arm vein with an oiled syringe to a waxed tube containing 10 0 ml of a saturated solution of sedormid in saline   The contents of the tube were immediately mixed by inversion and 2 5 ml transferred at minute intervals in a waxed pipette to clot retraction tubes each of which contained 4 units of thrombin in 0 1 ml of saline   Clot retraction in each tube was measured   In addition, dry films, which were later stained with Leishman's stain, were made at minute intervals from a similar preparation made at the same time, and also from a further sample of blood diluted equally with saline   This was done so that the changes in the platelets could be accurately correlated with the changes observed in clot retraction   These experiments were performed on Cases I and II and the results are given in Figs 3 and 4 They show that, in both cases, when coagulation was delayed only by the use of waxed vessels, thrombin lost its capacity to antagonise the action of sedormid on clot retraction within two minutes   In Case II this corresponded with the onset of platelet lysis by sedormid, but in Case I lysis was not

observed before the blood clotted spontaneously ten minutes after exposure to the drug These results show that there is no correlation between the time taken by sedormid to cause lysis of platelets and the time at which thrombin loses its power to antagonise the action of sedormid on clot retraction, although it must be emphasised that sedormid may well produce
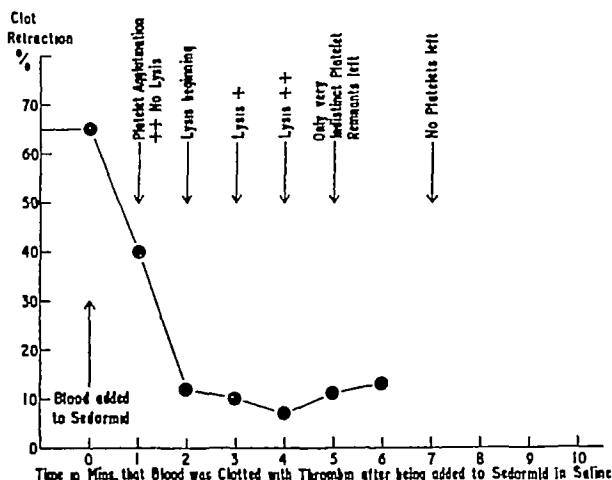
Fig 4   Graph showing the effect of thrombin on the inhibition of clot retraction by a saturated solution of sedormid in saline and the action of sedormid on the platelets in a similar preparation made at the same time   Blood taken from Case II (M B)   In the control preparations containing saline alone platelet lysis was only just detectable at 7 minutes As in Table VI, the term lysis refers only to morphological changes in the platelets

changes in the platelets which can interfere with their capacity to promote clot retraction long before it causes platelet lysis   It seems probable that the clot retraction values obtained in these experiments are the resultant of two different processes acting on the platelets, namely, the normal changes in the platelets which occur during coagulation and which are probably accelerated when coagulation is accelerated by thrombin, and the action of sedormid in causing abnormal platelet lysis   If thrombin is added to the blood sufficiently early in the experiment then the normal process of coagulation can probably so alter the platelets immunologically before sedormid has time to exert its full effect upon them that clot retraction approaches normal, but when thrombin is added later the action of sedormid on the platelets is by then so advanced (although it may not yet be detectable microscopically) that the platelets are incapable of promoting normal clot retraction

Inhibitory effect of sodium citrate on the action of sedormid on clot retraction

In the experiments reported above, in which the effect of thrombin on the action of sedormid on clot retraction was investigated, the antagonistic action of thrombin was found to be much less when the sedormid was dissolved in saline than when a saturated solution of sedormid in sodium

TABLE VII

Effect of sodium citrate on the inhibition of clot retraction by sedormid in blood of sedormid sensitive patients

Patient (1)	Details of experiment (2)	Coagulant (3)	Time coagulant added after blood or plasma mixed with sedormid solution (4)	No of determinations for each experiment (5)	Clot retraction %		Platelet lysis by sedormid during coagulation (8)
					Without sedormid (6)	With solutions in Col 2 saturated with sedormid (7)	
Case I (N.H)	2 0 ml. blood + 0 5 ml citrate saline A*	0 2 ml 1 18% CaCl₂	5 mins	4	64	19	
	2 0 ml blood + 0 5 ml citrate saline B*	0 3 ml 1 18% CaCl₂	5 mins	4	64	25	
	1 5 ml blood + 0 4 ml 3 2% sodium citrate	4 units thrombin in 0 1 ml saline	15 mins	4	70	67	
	2 0 ml plasma† + 0 5 ml saline	0 8 ml 1 18% CaCl₂	30 mins	4	83	38	
	Ditto	Ditto	30 mins	4	70	<4	

Case II (M B)						
2 0 ml blood + 0 5 ml citrate saline A*	4 units thrombin in 0 1 ml saline	5 mins	4	66	10	
2 0 ml blood + 0 5 ml citrate saline B*	Ditto	20 mins	4	64	13	+
2 0 ml blood + 0 5 ml citrate saline C*	Ditto	20 mins	4	66	18	+
2 0 ml blood + 0 15 ml 3 2% sodium citrate	0 6 ml 1 18% CaCl₂	5 hrs	2	52	27	
2 0 ml blood + 0 5 ml 3 2% sodium citrate	4 units thrombin in 0 1 ml saline	2 hrs	4	70	65	Lysis definite but many platelets appear un affected
Ditto	0 6 ml 1 18% CaCl₂	5 hrs	2	58	41	
1 5 ml plasma† + 0 5 ml saline	0 5 ml 1 18% CaCl₂	20 mins	1	83	6	
2 0 ml plasma† + 0 5 ml saline	4 units thrombin in 0 1 ml saline	30 mins	4	80	<4	+

* The compositions of the various preparations of citrate saline are as follows —

A   0 37% sodium citrate in 0 88% sodium chloride

B   0 8% sodium citrate in 0 7% sodium chloride

C   0 06% sodium citrate in 0 63% sodium chloride   This corresponds with a concentration of 0 15 ml 3 2% sodium citrate to 2 0 ml blood and is the concentration of citrate used in preparing the samples of plasma referred to in this table

† Plasma prepared by citrating blood in proportion 0 15 ml 3 2% sodium citrate to 2 0 ml blood and then centrifuging slowly to precipitate the R & W B Cs whilst leaving the platelets in suspension

citrate was used (*compare* Tables IV and V) unless the sedormid in sodium citrate was allowed to act for 5 minutes before the thrombin was added Thus, when the blood was added to a mixture of thrombin and sedormid in saline clot retraction was increased by only 14% in Case I and 19% in Case II as compared with the clot retraction obtained when blood was added to a saturated solution of sedormid in saline alone   When, however, the blood was added to a solution of thrombin in a saturated solution of sedormid in citrate saline, clot retraction was increased on an average by as much as 31% in Case I and 43% in Case II as compared with the clot retraction of blood added to a saturated solution of sedormid in citrate saline to which thrombin was added after 5 minutes   These findings suggest that the sodium citrate may have delayed the action of sedormid and so enabled the thrombin to carry the coagulation process further before sedormid could exert its effect and that in this way the thrombin was able to reduce the action of the drug on clot retraction to a much greater extent than when the drug was dissolved in saline alone

Further evidence that sodium citrate interferes with the action of sedormid on clot retraction was obtained when an attempt was made to demonstrate the action of sedormid on the clot retraction of blood containing an excess of this substance (*see* Table VII)   When blood from Case I was mixed with a saturated solution of sedormid in 3 2% sodium citrate in the proportion of 0 4 ml of sedormid solution to 1 5 ml of blood and clotted 15 minutes later, the clot retraction was not significantly lower than that observed when 3 2% sodium citrate alone was used   This experiment was repeated with blood from Case II, using 0 5 ml of citrate to 2 0 ml of blood On this occasion the blood was allowed to stand on the bench for several hours after it had been mixed with citrate or with a saturated solution of sedormid in citrate before being clotted because it was felt that this might give the sedormid time to act if its action was not completely inhibited, but merely delayed, by the presence of the anticoagulant   After a delay of two hours sedormid still did not cause a significant reduction in clot retraction but when the sedormid was allowed to act for 5 hours before the blood was clotted a small but significant reduction in clot retraction was obtained although this was much less than that obtained when blood was added to a saturated solution of sedormid in saline

These results are in marked contrast to those obtained in the experiments in which 0 37% and 0 8% sodium citrate in saline were used in the proportion of 0 5 ml of citrate solution to 2 0 ml of blood   The action of sedormid was well preserved with either of these preparations   Neither, however, contained sufficient sodium citrate to prevent clotting in unwaxed vessels on all occasions although the preparation containing 0 8% sodium citrate would often do so   The lowest concentration which would prevent coagulation on all occasions and yet preserve the action of sedormid was found to be 0 96% sodium citrate ($Na_3C_6H_5O_7$ $2H_2O$) using

0 5 ml of 0 96% sodium citrate in 0 63% sodium chloride to 2 0 ml of blood
This is equivalent to using 0 15 ml of isotonic (3 2%) sodium citrate to 2 0 ml
of blood   The action of sedormid on the retraction of plasma clots was
found to be readily demonstrable if the plasma was prepared by citrating
blood in this way, and then centrifuging at 2,500 r p m for 4 minutes, to
precipitate the red and white cells whilst leaving the platelets in suspension
This concentration of citrate has been used in all the experiments on plasma
reported below

The platelets were observed during coagulation in some of the
experiments on Case II   In three preparations, in which sedormid reduced
clot retraction, the platelets underwent abnormally rapid lysis but in one
experiment, in which sufficient sodium citrate was used to prevent the action
of sedormid on clot retraction, although some of the platelets underwent
lysis, the majority appeared unaffected   It therefore seems probable that
sodium citrate interferes with the effect of sedormid on clot retraction by
exerting a protective action on the platelets

The effect of the addition of normal platelets to whole blood on the inhibition of
clot retraction by sedormid

It was by now clear that sedormid causes lysis of platelets during the
coagulation of the blood of patients who have recovered from sedormid
purpura and it appeared probable that this is the mechanism by which
sedormid reduces clot retraction   It therefore seemed likely that the addition
of normal platelets to the blood of a patient who was sensitive to sedormid
would restore normal clot retraction in the presence of the drug, because
these normal platelets would persist and would be able to promote normal
clot retraction even though the platelets originally present had been destroyed

Before the effect of the addition of normal platelets on the action of
sedormid on clot retraction could be investigated it was necessary to establish
that this procedure had no effect on normal clot retraction   A suspension
in 3 2% sodium citrate, of normal platelets, isolated by a method which is
described below, was therefore added to a series of samples of normal blood
and to samples of blood from Cases I and II   These preparations were
clotted with either thrombin or isotonic calcium chloride and the clot
retraction was compared with that of blood diluted with an equal volume of
sodium citrate alone and clotted in the same way   In every case but one,
the ability of the platelet suspension to restore clot retraction to plasma,
the clot retraction of which had been abolished by centrifuging out the
platelets was also investigated using thrombin as a coagulant, and shown to
be normal *   The results of these experiments, which are given in Table VIII,
show that in all the cases investigated the addition of normal platelets to
whole blood, to produce a final concentration of from 170,000 per c mm to
420,000 per c mm of added platelets in the mixture of blood and platelet
suspension, had no significant effect on clot retraction

* *See* footnote page 251

TABLE VIII

Effect on clot retraction of the addition of normal human platelets to blood of individuals showing no abnormalities of coagulation, and to the blood of 2 patients who had recovered from scleromid purpura

Patient.	Diagnosis.	Expt. No	Details of experiment.	Platelet suspension Counts per c mm			Clot retraction of blood			Platelet count of platelet free plasma per c mm	Clot retraction of platelet free plasma used for testing platelet suspensions		
				Platelets ×10³	R B O	W B O	No of determinations for each expt	Retraction % without platelets.	Retraction % with added platelets.		No of determinations for each expt	Retraction % without platelets.	Retraction % with added platelets.
P O	Chronic aleukaemic myeloid leukaemia	1	2 0 ml blood or platelet free plasma + 0 1 ml platelet suspension in 3 2% sodium citrate + 4 units thrombin in 0 1 ml saline	2 5	1 30	500	1	75	70	—	—	—	—
T H	Healthy man	2	2 0 ml blood or platelet free plasma + 0 1 ml platelet suspension in 3 2% sodium citrate + 0 6 ml saline + 0 15 ml 1 18% calcium chloride (In the experiments on plasma, 4 units thrombin in 0 1 ml saline was used in place of the calcium chloride)	4 3	120	240	1	72	71	1,300	4	<1	83
P L	ditto	3					1	66	67				
O O	ditto	4					1	63	62				
H S	ditto	5					1	68	65				
D I	ditto	6					1	55	60				
N H	Case I	7	1 5 ml blood or platelet free plasma + 0 1 ml platelet suspension in 3 2% sodium citrate + 4 units thrombin in 0 1 ml saline	0 0	2,500	250	1	70	71	—	3	<1	05
M B	Case II	8	As for experiments 2-6	3 0	1,000	250	1	64	65	3,160	4	<1	01

A suspension of normal platelets in 3 2% sodium citrate was subsequently added to the blood of Case II (M B) and the effect of sedormid on its clot retraction was observed   As a control, the effect of sedormid on the clot retraction of a further sample of this patient's blood diluted with 3 2% sodium citrate alone was investigated   All the samples were subsequently clotted with isotonic (1 18%) calcium chloride   The results are shown in Table IX   The control experiments showed that the quantity of sodium citrate used did not interfere with the action of sedormid in practically abolishing the clot retraction of this patient's blood   When the same volume of a suspension of normal platelets in sodium citrate was added to this patient's blood to produce a final concentration of added platelets of about 140,000 per c mm   in the mixture of blood and platelet suspension, there was no improvement in clot retraction in the presence of sedormid although the addition of this number of platelets permitted almost normal clot retraction in the absence of the drug   That the platelets added were normal was shown by adding an equal concentration of the platelet suspension to the same patient's plasma after its clot retraction had been abolished by centrifuging out the platelets   This raised its clot retraction from less than 4% to over 80% when thrombin was used as a coagulant *   It was therefore clear that the addition of normal platelets in the concentration used did not improve the clot retraction of this patient's blood in the presence of sedormid †

Evidence that the effect of sedormid in reducing clot retraction is due to the action of a factor in the plasma

The result of the above experiment was surprising and seemed to indicate either that there is some other factor, in addition to platelet lysis, which is responsible for the reduction of clot retraction by sedormid, or alternatively, that the mechanism responsible for the lysis of these patients' platelets is also capable of inactivating normal platelets   To investigate this further, normal platelets were suspended in the plasma of patients who had recovered from sedormid purpura after the platelets originally present in the plasma had been removed by centrifugation, and the platelets of such patients were suspended in normal plasma freed of platelets in the same way   The effect of sedormid on the clot retraction of these preparations was then investigated, and finally the effect of this substance on the platelets during coagulation was observed in a hæmocytometer chamber   These experiments proved

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* Thrombin was used in preference to calcium chloride in these control experiments because the clot retraction of citrated platelet rich plasma clotted with calcium chloride is abnormally low as compared with that of normal plasma, separated without anti coagulants and allowed to clot spontaneously   Such samples of plasma always retract to over 80%, as do preparations of normal citrated plasma when clotted with thrombin   The use of thrombin therefore made it easier to be certain that the clot retraction of the platelet free " plasma to which platelets had been added, was normal   Thrombin was not used, however, in investigating the effect of the addition of normal platelets on the action of sedormid on the clot retraction of whole blood because it would have confused the issue if more than one normal factor has been added at the same time

† It was subsequently found (*see* page 258 and Table XII) that when very high concentrations of platelets were used the action of sedormid could be abolished

TABLE IX

Effect of the addition of moderate numbers of normal platelets to whole blood on the inhibition of clot retraction by serotonin in Case II (M B)

Details of experiment	Clot retraction			
	After addition of 0 5 ml serotonin in saline		After addition of 0 5 ml saline	
	No of expts		No of expts	
0 15 ml 1 18% calcium chloride + 0 1 ml 3 2% sodium citrate + 2 0 ml blood	2	less than 4%	2	59%
0 15 ml 1 18% calcium chloride + 0 1 ml platelet suspension* in 3 2% sodium citrate + 2 0 ml blood	3	less than 4%	4	47%
	No of expts		No of expts	Clot retraction
2 0 ml platelet "free" plasma from Case II + 0 5 ml saline + 0 1 ml 3 2% sodium citrate + 0 1 ml saline containing 4 units thrombin	2	less than 4%	2	less than 4%
2 0 ml platelet "free" plasma from Case II + 0 5 ml saline + 0 1 ml platelet suspension* in 3 2% sodium citrate + 0 1 ml saline containing 4 units thrombin	2		2	81%

* The platelet suspension had the following composition —

Platelets	2,870,000/c mm
R B C	10,000/c mm
W.B C	350/c mm

difficult and there were many failures   The failures were of two types
First, when no clot retraction was obtained, even in the platelet containing
preparations to which no sedormid had been added, and second, when normal
clot retraction was obtained even in those tubes in which the platelets of a
sensitised patient had been added to her own plasma which had been
subsequently clotted in the presence of sedormid   The first type of failure
appeared to be due to the physical effect of the manipulations required to
isolate the platelets for it has been shown by Tocantins (7) that even shaking
platelet suspensions may destroy their capacity to promote normal clot
retraction   Failures of this type became infrequent as the method of isolating
platelets, referred to below, was improved   Sometimes, however, adhesion
of the clot to the walls of the tube prevented retraction   The danger of
loss of retraction from adhesion in these preparations is much greater than
with normal plasma clots and even lining the tubes with a layer of paraffin
wax may not be sufficient to permit normal retraction because clot retraction,
after removal and resuspension of platelets, is less powerful than with normal
plasma   This is probably due to injury to the platelets during the
manipulations involved   For this reason, in some of these experiments, the
tubes were lined with paraffin wax and subsequently, just before use, were
rinsed out with liquid paraffin   During the incubation of such tubes, the
paraffin wax dissolves in the liquid paraffin, forming a fluid layer to which
adhesion cannot occur

Failure of the second type, in which normal clot retraction was obtained
even when the platelets of a sensitised patient were suspended in her own
plasma and clotted in the presence of sedormid, was more difficult to explain
It was not due to the use of an excess of anticoagulant because in all these
experiments the amount used (0 075 ml of 3 2% sodium citrate per 1 0 ml of
blood) had previously been shown not to prevent the action of sedormid
on clot retraction   It was thought at first that it might be due to the
inactivation of some thermolabile factor concerned in the lysis of platelets
by sedormid and that this factor might be destroyed during the manipula-
tions involved   For this reason, except when they were being centrifuged,
all the preparations were kept in the refrigerator at about 4°C until they
were finally mixed with sedormid or saline   They were then left on the
bench for half an hour to permit the interaction of sedormid with the plasma
constituents before being clotted   These precautions were, however,
insufficient to preserve the action of sedormid on clot retraction and no
further progress was made until it was realised that if an excess of platelets
was present in the final preparations of plasma, then sedormid caused only
incomplete lysis and many platelets were left which were able to promote
normal clot retraction   The optimal number of platelets was not accurately
determined but appeared to be of the order of 300,000 per c mm to 400,000
per c mm in plasma   When all these precautions were taken, satisfactory
results were almost invariably obtained

TABLE X

The effect on the inhibition of clot retraction by sedormid of the addition of normal platelets to the platelet "free" plasma of patients who have recovered from sedormid purpura, and of the addition of the platelets of such patients to normal platelet "free" plasma

Expt No	Details of experiments on platelet "free" plasma to which platelets added	Case I (N H)					Case II (M B)				
		Coagulant	No of determinations for each expt	Clot retraction %		Platelet lysis by sedormid during coagulation	Coagulant	No of determinations for each expt	Clot retraction %		Platelet lysis by sedormid during coagulation
				Diluted with 0 5 ml saline	Diluted with 0 5 ml sedormid in saline				Diluted with 0 5 ml saline	Diluted with 0 5 ml sedormid in saline	
1	2 0 ml platelet "free" plasma from Case I or II + normal platelets	0 8 ml 1 18% CaCl₂	4	50	16	+	1 unit thrombin 0 1 ml saline	4	90	<4	+
2	2 0 ml normal platelet "free" plasma + normal platelets		4	42	47	0		4	90	92	0
3	2 0 ml normal platelet "free" plasma + platelets from Case I or II		4	30	37	0		4	94	96	0
4	2 0 ml platelet "free" plasma from Case I or II + platelets from the same patient		4	14	<4	+		1	93	<4	+

Details of experiments on platelet "free" plasma	Coagulant	No of expts	Clot retraction %	Coagulant	No of expts	Clot retraction %
2 0 ml platelet "free" plasma used in expt 1 + 0 5 ml saline		4	<4		4	<4
2 0 ml platelet "free" plasma used in expt 2 + 0 5 ml saline	0 8 ml 1 18% CaCl₂	4	<4	1 unit thrombin in 0 1 ml saline	4	<4
2 0 ml platelet "free" plasma used in expt 3 + 0 5 ml saline		4	<4		4	<4
2 0 ml platelet "free" plasma used in expt 4 + 0 5 ml saline		4	<4		4	<4

Platelet "free" plasma in these experiments was prepared by centrifuging blood citrated with 0 75 ml 3 2% sodium citrate to 100 ml blood   The counts performed on the different samples of plasma are given in Table XI

These experiments were performed on Cases I and II   The details of the method by which the platelets were isolated, both for these and for the preceding experiments, will be given in a subsequent communication   The following is a brief description   Oxalated or citrated blood is centrifuged slowly at such a speed that the red and white cells are precipitated, while the platelets are left suspended in the plasma   This plasma is siphoned off and centrifuged at high speed to precipitate the platelets   The supernatant plasma is discarded and the platelets resuspended in isotonic potassium oxalate or sodium citrate   They are again precipitated by centrifuging and the supernatant fluid discarded   The platelets are washed twice more in this way   They may then be resuspended in a very small volume of citrate, as in the experiments reported above and added to blood or plasma in that state or they may be resuspended in platelet " free " plasma, as in the experiments reported below, either immediately, or after they have been further washed in saline   In every case platelets were prepared from blood

TABLE XI

Counts performed on samples of plasma referred to in Table X

	Case I (N H) (counts per c mm)				Case II (M B) (counts per c mm)			
	Expt 1	Expt 2	Expt 3	Expt 4	Expt 1	Expt 2	Expt 3	Expt 4
Platelet count on platelet " free " plasma	20,000	40,000	55,000	20,000	550	450	1,650	1,850
Platelet count on platelet " free " plasma after addition of platelets	560,000	620,000	470,000	560,000	220,000	180,000	395,000	400,000
R B C count on platelet " free " plasma after addition of platelets	380	480	7,000	6,000	1,000	1,200	5	10
W B C count on platelet " free " plasma after addition of platelets	40	20	200	100	50	40	10	5

of the same A B O group as that of the plasma in which they were to be suspended   In the experiments on Case I the final washing was done with 3 2% sodium citrate and in those on Case II, with saline   The samples of plasma used in the experiments on Case I were clotted with isotonic calcium chloride and those used in the experiments on Case II, with thrombin These small variations in technique appear to have made no significant

difference to the results which were identical in type in the two patients and may be considered together   They are shown in detail in Tables X and XI and illustrated in Figs 5 and 6

In each experiment it was shown that clots formed from the platelet " free " preparations of plasma were irretractile and that normal retractility was restored by the addition of platelets, thus showing that the platelets had not been inactivated during the process of isolation   Also, the platelets and plasma referred to as " normal " were investigated (Experiment 2, Table X) and it was shown that sedormid had no effect on the clot retraction of such samples of plasma to which " normal " platelets had been added

It will be seen from Experiment 4 that, as has already been stated, the effect of sedormid on the clot retraction of plasma of patients who have recovered from sedormid purpura was not abolished by removing the platelets by centrifugation and then replacing them   This same reduction in clot retraction by sedormid was also observed if these platelets were replaced by normal platelets (Experiment 1)   This finding seemed to confirm the results obtained in a previous experiment in which it was shown that the addition of normal platelets to the whole blood of a patient who had recovered from sedormid purpura failed to promote normal clot retraction in the presence of the drug   When, however, the platelets of a sensitised patient were added to normal platelet " free " plasma, sedormid had no effect on clot retraction (Experiment 3)   This seemed to demonstrate beyond doubt that the inhibition of clot retraction by sedormid is due to some factor in the plasma of patients who are sensitive to the drug, and not to an abnormality of the platelets themselves   Finally, the platelets in each preparation were observed during coagulation in a hæmocytometer chamber   Abnormal lysis of platelets occurred only in those experiments in which sedormid caused a reduction in clot retraction (Experiments 1 and 4)   It will be noted that these are the only experiments in which plasma from a patient who had recovered from sedormid purpura was used   Both homologous (Experiment 4) and heterologous (Experiment 1) platelets underwent lysis in such patients' plasma in the presence of sedormid   No lysis of the platelets of such patients, however, was observed when these were suspended in normal plasma and subsequently clotted in the presence of sedormid   (Experiment 3)

Evidence that the effect of sedormid on clot retraction is due solely to its lytic action on platelets during coagulation

The above experiments suggest strongly that platelet lysis during coagulation is the only factor involved in the inhibition of clot retraction by sedormid   These experiments, however, involved the use of an anticoagulant and consequently necessitated promoting coagulation with either calcium chloride or thrombin   Although these substances, in the concentrations used, did not appear to modify the action of sedormid significantly, it seemed desirable to confirm that platelets were indeed the only factor involved, by a

method which did not require the use of anticoagulants   A procedure by which this might be done was suggested by the observation, mentioned above, that if a large excess of platelets was added to the plasma of a sensitised patient, not all the platelets underwent lysis when the plasma was clotted in the presence of sedormid and that the surviving, unaffected platelets were able to promote normal clot retraction in the presence of the drug   It seemed probable that if a high concentration of platelets, much greater than that employed in the experiment summarised in Table IX, was used and if the platelets were suspended in saline, and not in citrate, then it might be possible to promote normal clot retraction in the blood of sensitised patients in the presence of sedormid by the addition of platelets alone   Suspensions of platelets in saline, or sedormid in saline, containing up to 3,500,000 platelets per c mm were therefore added to whole blood and the effect of the sedormid on the clot retraction of these preparations was compared with that of sedormid in saline on the clot retraction of blood to which no platelets had been added   The results of these experiments, which were performed on Case II, are shown in Table XII   When a suspension containing 1,400,000 platelets per c mm was used the clot retraction in the presence of sedormid was increased from less than 4%, in the tubes to which no platelets had been added, to 55% in the tubes containing the platelet suspension   This latter figure is only 9% less than the retraction in the tubes to which platelets but no sedormid had been added   That is, the reduction in clot retraction by sedormid was decreased from over 58% to 9% by the addition of the platelets   This experiment was later repeated using over twice the number of added platelets   With such a very high concentration of platelets some allowance had to be made for the volume of platelets added   To compensate for the dilution of the sedormid solution by platelets, the control preparations containing sedormid but no platelets were diluted with saline in the proportion of 0 1 ml of saline to 0 4 ml of a saturated solution of sedormid in saline   The platelet suspension used on this occasion contained 3,500,000 platelets per c mm and raised the platelet count in the blood from its initial level of 490,000 per c mm to 1,375,000 per c mm in terms of the original volume of whole blood   Raising the platelet count to this level completely abolished the action of sedormid on clot retraction   As no other clotting factor except washed platelets, and no anticoagulants were used, these results seemed to prove conclusively that platelet lysis is the only factor involved in the loss of clot retraction which is observed when the blood of sensitised patients is allowed to clot in the presence of sedormid

To conclude, therefore, it appears clear that sedormid reduces the clot retraction of the blood of patients who have recovered from sedormid purpura by causing abnormally rapid lysis of the platelets during coagulation   This platelet lysis is the only factor involved and it results from the action of some factor in the plasma of these patients and is not due to any peculiarity of the

TABLE XII

Effect of the addition of very large numbers of normal platelets on the inhibition of clot retraction by sedormid in blood from Case II (M B)

Date	Details of experiment	Platelets × 10³	Platelet suspensions in saline. Counts per c.mm.		Clot retraction of blood			Platelet count on blood before platelets added. Count per c.mm.	Platelet count on platelet free plasma per c.mm	Clot retraction of platelet free plasma used for testing platelet suspensions.		
			R B C	W B C	No. of determinations for each expt.	Retraction % without added platelets	Retraction % with added platelets			No. of determinations for each expt.	Retraction % without added platelets	Retraction % with added platelets
20 6 19	2·0 ml. blood + 0·5 mL. saline or platelet suspension in saline	1 6c	310	60	4	6~	64	—	1,000	4	<1	90
	0 ml. blood + 0·5 ml. sedormid in saline or platelet suspension in sedormid in saline	1 11	110	30	1	<1	36					
18 6 19	2·0 ml. blood + 0·5 ml. saline or platelet suspension in saline	3 40	1,200	200	1	68	68	190 000	500	4	<1	95
	2·0 ml. blood + 0·1 ml. saline + 0·4 ml. sedormid in saline or 2·0 ml. blood + 0·5 ml. platelet suspension in sedormid in saline	3 64	1,800	100	4	<1	68					

platelets themselves   It also seems clear that the lysis of platelets is a nonspecific effect, and that the thrombocytolysin acts equally readily on the platelets of patients who are not sensitive to sedormid

Summary

1   The action of sedormid in reducing clot retraction in blood of patients who have recovered from sedormid purpura has been investigated by adding fibrinogen, thrombin and platelets to different samples of the blood of such patients, and investigating the action of sedormid on the clot retraction of the resulting mixtures   This was done on the supposition that a normal clotting factor might replace one sensitive to sedormid and so permit normal clot retraction in the presence of the drug

2   The addition of fibrinogen to the blood of such patients, or to normal blood, reduces clot retraction   When sedormid and fibrinogen are added to the blood of sedormid sensitive patients the reduction in clot retraction is greater than with either alone

3   When thrombin is added to normal blood or to the blood of patients who have recovered from sedormid purpura, clot retraction is generally slightly reduced   When thrombin and sedormid are added simultaneously to the blood of such patients the thrombin lessens the reduction in clot retraction due to the sedormid but if the sedormid is allowed to act for 5 minutes before the thrombin is added, then the action of sedormid in reducing clot retraction is unimpaired

4   These experiments, therefore, provide no evidence that either fibrinogen or thrombin is concerned in the action of sedormid on clot retraction   The action of thrombin appears to be merely that it accelerates coagulation and so prevents sedormid from acting as completely as usual

5   If the blood of patients who have recovered from sedormid purpura is observed microscopically during coagulation in the presence of sedormid the platelets are seen to undergo abnormally rapid lysis   This almost certainly explains the action of sedormid in reducing clot retraction because, if platelets are removed from blood, clot retraction is invariably abolished

6   The presence of an excess of sodium citrate prevents the action of sedormid on clot retraction   It appears to do this by protecting the platelets from abnormal lysis by sedormid during coagulation

7   The addition of normal platelets to normal blood or to the blood of sedormid sensitive patients has no effect on clot retraction

8   The addition of normal platelets to the blood of a sedormid sensitive patient to produce a final concentration of about 140,000 per c mm of added platelets does not improve clot retraction in the presence of sedormid although this concentration of platelets is sufficient to restore normal clot retraction to this patient's plasma after its clot retraction has been abolished by centrifuging out the platelets

9   If normal platelets are added to the platelet " free " plasma of patients who have recovered from sedormid purpura and the plasma is subsequently clotted in the presence of sedormid, the platelets are lysed abnormally rapidly and clot retraction is greatly reduced

10   If the platelets of sedormid sensitive patients are added to normal platelet " free " plasma which is then clotted in the presence of sedormid, the platelets are not lysed abnormally rapidly and clot retraction is normal

11   If an excess of platelets is added to the plasma of a sensitised individual which is then clotted in the presence of sedormid not all the platelets undergo the abnormal lysis due to the drug and those remaining are able to promote normal clot retraction   If a sufficiently large excess of platelets is added to whole blood the action of sedormid on clot retraction is entirely abolished

12   It is therefore concluded that sedormid reduces clot retraction in the blood of patients who have recovered from sedormid purpura by causing lysis of platelets during coagulation and that this is the only factor involved   This platelet lysis results from the action of some factor in the plasma and is not due to any peculiarity of the platelets themselves

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(a)

(a)   (b)
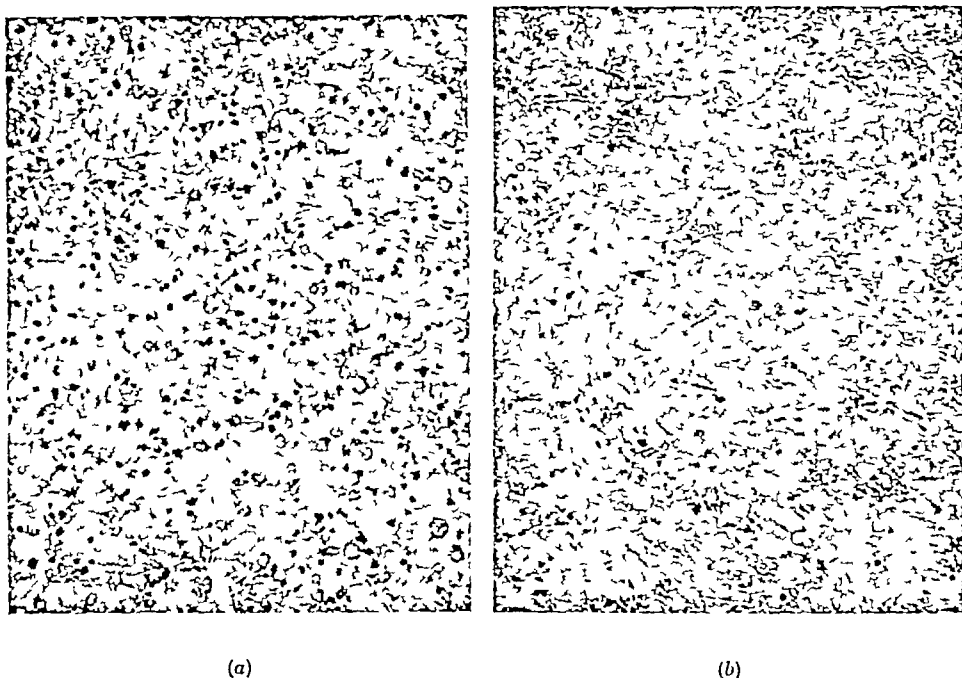
Fig 2   Photomicrographs showing lysis of platelets by sedormid during coagulation, in a
hæmocytometer chamber, of platelet rich plasma from a patient (Case II (M B)) who has
recovered from sedormid purpura   Each photograph was taken 20 minutes after the
sedormid or saline was added to the plasma

   (a) Plasma diluted with saline   Clot contains numerous platelets
   (b) Plasma diluted with a saturated solution of sedormid in saline
        The platelets in the clot have practically all undergone lysis
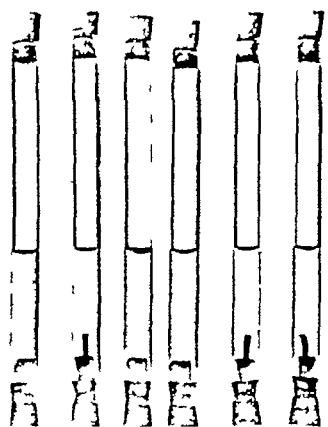
(a)   (b)   (c) (d)   (e)   (f)   (a)   (b)   (c) (d)   (e)   (f)
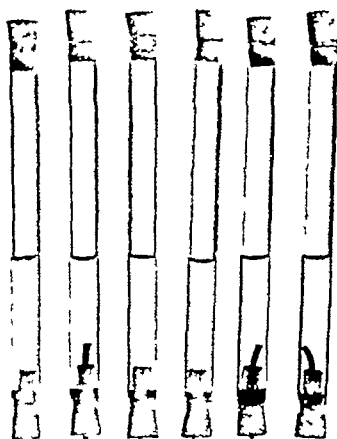
Fig 5   Fig 6

Fig 5   Effect on the action of sedormid on clot retraction of the addition of normal platelets to the platelet   free ' plasma of a patient (Case II (M B)) who has recovered from sedormid purpura

(a) Platelet " free ' plasma from Case II plus normal saline   Clot retraction nil

(b) Platelet " free ' plasma from Case II plus normal platelets plus saline   Clot retraction normal

(c) Platelet ' free " plasma from Case II plus normal platelets plus a saturated solution of sedormid in saline   Clot retraction nil

(d) Normal platelet " free " plasma plus saline   Clot retraction nil

(e) Normal platelet   free " plasma plus normal platelets plus saline   Clot retraction normal

(f) Normal platelet ' free " plasma plus normal platelets plus a saturated solution of sedormid in saline   Clot retraction normal

Fig 6   Effect on the action of sedormid on clot retraction of the addition of platelets from a patient (Case II (M B)) who has recovered from sedormid purpura, to normal platelet ' free ' plasma

(a) Platelet ' free " plasma from Case II plus saline   Clot retraction nil

(b) Platelet " free " plasma from Case II plus platelets from Case II plus saline   Clot retraction normal

(c) Platelet ' free   plasma from Case II plus platelets from Case II plus a saturated solution of sedormid in saline   Clot retraction nil

(d) Normal platelet " free " plasma plus saline   Clot retraction nil

(e) Normal platelet   free   plasma plus platelets from Case II plus saline   Clot retraction normal

(f) Normal platelet   free ' plasma plus platelets from Case II plus a saturated solution of sedormid in saline   Clot retraction normal

THE CAUSE OF THROMBOCYTOPENIA IN SEDORMID PURPURA

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IF a patient who has recovered from sedormid purpura is given a test dose of sedormid by mouth, the platelet count falls rapidly and may reach extremely low levels within 30 minutes to an hour (8) The speed with which the count falls suggests that the platelets must be destroyed in the peripheral blood, for an effect on the bone marrow, by itself, would hardly be capable of producing so rapid a reduction in the number of platelets It has been shown that sedormid causes agglutination of platelets when the blood of some of these patients is mixed with ten times its volume of a saturated solution of sedormid in 3 2% sodium citrate although the platelets in such preparations do not undergo lysis even after 4 to 5 hours (1) An abnormally rapid lysis of platelets, which is associated with a marked reduction in clot retraction, does, however, occur when the blood of these patients is allowed to clot in the presence of sedormid (1, 2) The effect of sedormid on clot retraction can be demonstrated on citrated blood clotted with either calcium chloride or thrombin if minimal concentrations of sodium citrate are used, but is inhibited in the presence of an excess of sodium citrate The sodium citrate appears to act by reducing greatly the action of sedormid in causing abnormal platelet lysis during coagulation (2) It seemed therefore that the failure to demonstrate platelet lysis in citrated blood, referred to above, might well have been due to the fact that a large excess of sodium citrate was used in these experiments and that this, while permitting platelet agglutination, protected the platelets from the lytic action of the drug The effect of sodium citrate and other anticoagulants on the action of sedormid on the platelets of these patients was therefore investigated It is the purpose of this paper to show that sedormid causes agglutination and lysis of the platelets of sedormid sensitive patients but that platelet lysis is inhibited by an excess of any of the anticoagulants

used   In addition, an attempt has been made to discover which components of the sedormid molecule are principally concerned in the action of sedormid on the platelets

The patients investigated, N H (Case I), M B (Case II) and J MacP (Case III) are the same as those referred to in previous reports on sedormid purpura (1, 2)

Methods

Platelet counts

These were done by one of the two following methods —

Modified Gram-Thomsen method (6, 9)   A known volume of isotonic sodium citrate or other suitable anticoagulant is placed in a waxed tube and a measured volume of blood, taken in an oiled syringe, is added   The tube is closed with a waxed cork and mixed by repeated inversion   A small volume of the diluted blood is transferred to a Wintrobe hæmatocrit tube (11) and the packed cell volume determined after centrifugation   The tube of diluted blood is then allowed to stand to permit sedimentation of the red and white cells   The platelets in such preparations remain uniformly suspended in the plasma for many hours   A sample of this plasma is diluted 1 100 with isotonic sodium citrate and mixed in a mechanical shaker for 5 minutes   A drop of this diluted plasma is transferred to a hæmocytometer chamber   The preparation is then allowed to stand for 20 minutes to permit the platelets to settle before they are counted   As the volumes of blood and anticoagulant originally used and the packed cell volume of the diluted blood are known, the number of platelets per c mm of whole blood can readily be calculated   In the work reported below, to ensure as great accuracy as possible, very large numbers of platelets, often over a thousand, have been counted in each preparation

Modified Tocantins method (10)   In this method the count is done immediately after the blood is mixed with anticoagulant and without allowing the red and white cells to sediment   The blood is further diluted 1   200 with isotonic sodium citrate and mixed for 5 minutes in a mechanical shaker   It is then transferred to a hæmocytometer chamber and the platelets are counted 20 minutes later   The main objection to this method is that the platelets tend to be obscured by the red and white cells present in the counting chamber   The method was, however, found useful for certain experiments in which the more accurate Gram-Thomsen method could not be used

Preparation of solutions

The method by which solutions of sedormid and chemically related substances were prepared has been described previously (1, 2)   Solutions of sodium citrate or potassium oxalate, which did not contain sedormid, have been boiled before use   The reason for this is given on p 272

*Agglutination and lysis of platelets by sedormid in blood diluted with a small
volume of an isotonic solution of sodium citrate*

The minimum quantity of sodium citrate which invariably prevents
coagulation of blood in unwaxed glass vessels has been shown to be
0 075 ml of 3 2% sodium citrate to 1 0 ml of blood   This concentration of
citrate permits the action of sedormid on clot retraction to be demonstrated
when either calcium chloride or thrombin are used as coagulants (2)
Blood, taken in oiled syringes, from patients who had recovered from sedormid
purpura was therefore mixed with a saturated solution of sedormid in 3 2%
sodium citrate in these proportions in waxed tubes   Samples were aspirated
at intervals of a minute and dry films were made which were subsequently
stained with Leishman's stain   Blood diluted with citrate alone in the
same proportions was used as a control

Films of blood taken from the three patients who had recovered from
sedormid purpura showed that in Cases I and II, platelet agglutination
began to appear at the end of the second minute after the blood was mixed
with the sedormid solution and became maximal 1 to 3 minutes later   By
this time the picture was very striking with many large agglutinates of
often as many as 20 to 30 platelets, very few platelets remaining free   A
typical picture is shown in Fig 1   In the same preparations it became
obvious after about 5 minutes in Case II and 10 minutes in Case I that the
platelets were beginning to undergo lysis   They began to swell and became
indistinct and many " ghost " forms were seen   Also, the agglutinates
were becoming smaller   This appeared to be due to separation of platelets
from the agglutinates as the number of free platelets was rising at the same
time   This last phenomenon had previously been observed in preparations
containing an excess of citrate (1)   The process of platelet lysis continued
rapidly for about 15 minutes after the blood was mixed with sedormid and
thereafter proceeded much more slowly   Even after 4 to 5 hours the process
was by no means complete and platelets were still fairly numerous although
they all showed striking morphological changes   Similar preparations were
made on two occasions with blood from Case III   In this case agglutination
of platelets did not occur until the end of the second hour and was never as
complete as in Cases I and II   Platelet lysis was just detectable at 2 hours
and was readily seen at 4 hours

These appearances were in striking contrast to those of the platelets
in blood diluted with citrate alone   In these preparations agglutination of
platelets was minimal even after 5 hours and the platelets at this time were
still numerous and appeared normal   A series of controls, mostly of blood
from healthy students, was also set up   Of 26 preparations, 25 showed no
significant agglutination of platelets in either sedormid or citrate   One,
presumably as a result of faulty technique, developed agglutination of the
platelets in both preparations

It therefore seemed clear that sedormid caused both agglutination and lysis of platelets in the blood of patients who had recovered from sedormid purpura   The degree of agglutination of the platelets in the sedormid preparations was, however, such that it was impossible to get a quantitative estimate of the number of surviving platelets by counting then in stained films by the method of Fonio (5)   During the course of these investigations it was noticed that if the tubes were allowed to stand so that the red and white cells sedimented leaving a layer of supernatant plasma, the plasma in the preparations containing citrate alone was opalescent but was quite clear in the preparations containing sedormid   The appearances of the plasma in two such tubes is shown in Fig 2   Microscopic examination showed that the opalescence of the citrate preparations was due to the presence of large numbers of platelets which were uniformly suspended in the plasma whereas only a few platelets were left suspended in the plasma in the sedormid preparations   The capacity of the platelets to remain suspended in plasma for several hours without sedimenting has been utilised by Gram (6) and Thomsen (9) in their very satisfactory method of platelet counting referred to above   It seemed that the application of this method to preparations of blood containing a saturated solution of sedormid in sodium citrate, and comparison with the results obtained with preparations of blood diluted with citrate alone might provide a simple way of assessing the percentage loss of free platelets after several hours' exposure to the action of sedormid

In order to show that sedormid had no such effect on the platelets of normal individuals, a series of controls, of blood from healthy individuals, was set up   In each case the blood was taken from an arm vein in an oiled syringe and 10 0 ml was added to 0 75 ml of a saturated solution of sedormid in 3 2% sodium citrate in a waxed tube which was then closed with a waxed cork and the contents mixed by inversion   An equal volume of blood was added to a similar tube containing citrate alone and treated in the same way   The two tubes were then stood for at least 5 hours, at the end of which time the red and white cells had sedimented leaving a supernatant column of plasma   Platelet counts were done on each specimen of plasma by the Gram-Thomsen method   On the first occasion on which this was done, a series of 10 control specimens of blood was investigated and in each the platelet count in sedormid was higher than that in citrate alone   It was then realised that although the sedormid containing solution had been boiled when the sedormid was dissolved in it, the citrate used as a control had not been boiled   It seemed probable that the slightly higher counts in the sedormid preparations were due to the small increase in the concentration of citrate resulting from the boiling of these preparations, for Wright (12) in a study of potassium oxalate, heparin, chlorazol pink and chlorazol blue has shown that the survival of platelets in such preparations, when relatively small amounts of anticoagulant are used, is proportional to the concentration of the anticoagulant   When both the citrate and sedormid in citrate solutions were boiled for the same length of time there was no significant difference

between the platelet counts in the two preparations in 10 normal controls (*see* Table I) It therefore seems clear that sedormid has no effect on the platelets of normal individuals Also, it would appear that the investigation, by this method, of the survival of free platelets in plasma in the presence of sedormid might be a simple and satisfactory test for sedormid sensitivity in patients who have recovered from thrombocytopenic purpura suspected of being due to this drug

TABLE I

Action of sedormid on the platelets of healthy individuals who were not hypersensitive to sedormid

Patient	Platelet count on plasma after 5 hours, expressed as platelets per c.mm of whole blood	
	0 075 ml 3 2% sodium citrate per 1 0 ml blood	0 075 ml 3 2% sodium citrate saturated with sedormid per 1 0 ml blood
H J	500,000	500 000
P McK	500,000	510,000
J T	480,000	490 000
E A	330,000	330,000
S A	460 000	440,000
P B	480,000	460 000
D S	240,000	260,000
L M	450 000	430,000
J G	410,000	380 000
L C	430,000	420,000

Action of anticoagulants on the agglutination and lysis of platelets by sedormid

Platelet counts were done by the Gram-Thomsen method using from 0 075 ml to 10 0 ml of a saturated solution of sedormid in isotonic (3 2%) sodium citrate to 1 0 ml of blood In each preparation the platelet count

was compared with that of an equal volume of blood diluted with the same quantity of 3 2% sodium citrate alone   Using a minimal quantity of 3 2% sodium citrate , namely, 0 075 ml of citrate solution to 1 0 ml of blood, the count in the sedormid preparations was about 70% less than in citrate alone in Case I and about 90% less in Case II   In Case III the reduction in free platelets was just over 40%   With increasing volumes of sodium citrate the percentage loss of free platelets became less and in Case I was 35% when 0 25 ml was used and only 15% when 0 5 ml of citrate solution was used to 1 0 ml of blood   With a proportion of 10 0 ml of citrate to 1 0 ml of blood, although there was slight agglutination of platelets in the sedormid preparation, there was virtually no loss of platelets in the plasma, the figure of 3% loss of platelets recorded for Case II being within the limits of experimental error by this method   Similar results were obtained with isotonic (1 6%) potassium oxalate and with solutions of heparin in saline   The action of sedormid on platelets was well shown when small quantities of either of these anticoagulants was used   With larger quantities both were able to inhibit the action of sedormid , completely in Case I, and almost completely in Case II   With sodium citrate and potassium oxalate, the quantity of the anticoagulant could only be increased by increasing the volume of solution used because increasing the concentration would have made the preparations hypertonic   This raised the problem whether the effect of large volumes of these anticoagulants on the action of sedormid on the platelets was due to the anticoagulant itself or was simply the result of the considerable dilution involved   To investigate this 1 0 ml of blood was added to 0 075 ml of 3 2% sodium citrate which had been diluted to 10 0 ml with saline and the platelet count by the Gram-Thomsen method was compared with that obtained when 1 0 ml of blood was diluted with 10 0 ml of the same solution saturated with sedormid   In both Cases I and II the reduction in the platelet count was approximately as great as when the blood was diluted with a saturated solution of sedormid in citrate alone in the proportion of 0 075 ml of citrate solution to 1 0 ml of blood, although there was practically no reduction in the count when 10 0 ml of sedormid in citrate was used to 1 0 ml of blood   Similar results were obtained when 1 0 ml of blood was added to 0 125 ml of a saturated solution of sedormid in 1 6% potassium oxalate which had been diluted to 10·0 ml with a saturated solution of sedormid in saline , the percentage loss of platelets being approximately the same as when a saturated solution of sedormid in potassium oxalate was used in the proportion 0 125 ml to 1 0 ml of blood It therefore seemed clear that the failure to demonstrate a reduction of free platelets in the plasma by sedormid when large volumes of these anticoagulants are used is due entirely to the anticoagulants themselves

With heparin it is, of course, possible to increase the concentration of the anticoagulant without significantly increasing its tonicity   It was found that large quantities of heparin in large or small volumes of saline

TABLE II

Effect of sodium citrate on the action of sedormid on the platelets of patients who have recovered from sedormid purpura

Patient	Date	Volume of 3 2% sodium citrate per 1 0 ml blood	Platelet count on plasma after 5 hours expressed as platelets per c.mm of whole blood		% loss of free platelets
			Without sedormid	With anti coagulant solution saturated with sedormid	
Case I (N.H)	21 10 47	0 075 ml	502,000	119,000	76 ⎫
	30 10 47		535,000	116,000	78 ⎪ 71
	11 11 47		481,000	169,000	65 ⎬
	29 1 48		648,000	223,000	66 ⎭
	21 10 47	0 25 ml	388,000	280,000	28 ⎫ 35
	11 11 47		512,000	304,000	41 ⎭
	30 10 47	0 5 ml	514 000	457,000	11 ⎫ 15
	11 11 47		464,000	379,000	18 ⎭
	11 11 47	1 0 0 ml	242 000	253 000	Nil
	13 1 48		354,000	371,000	Nil
	6 1 48	10 0 ml (0 075 ml citrate	364,000	90,000	75 ⎫
	13 1 48	diluted to 10 0 ml with	377,000	113,000	70 ⎬ 75
	3 2 48	saline)	360,000	76,000	79 ⎭
Case II (M B)	18 10 47	0 075 ml	300,000	34,000	91 ⎫
	10 1 48		300,000	22 000	93 ⎪ 93
	17 1 48		456,000	38,000	92 ⎬
	7 2 48		462,000	26,000	94 ⎭
	10 1 48	10 0 ml	407,000	395,000	3
	17 1 48	10 0 ml (0 075 ml citrate	365,000	87 000	76 ⎫ 80
	7 2 48	diluted to 10 0 ml with saline)	400,000	70,000	83 ⎭
Case III (J.McP)	23 1 48	0 075 ml	388,000	219,000	44

could antagonise the action of sedormid but that this action was well preserved if small quantities of heparin (8 to 16 units per 1 0 ml of blood) were used in either large or small volumes of saline thus showing that all the anticoagulants investigated can antagonise the action of sedormid on the platelets of patients who have recovered from sedormid purpura   The findings in all these investigations are summarised in Tables II, III and IV

It has been shown above that when sedormid is added to the blood of sensitised patients in the presence of minimal quantities of sodium citrate, the platelets are agglutinated and many also undergo lysis   Blood films made in the course of the above experiments showed that the same effects were produced when minimal quantities of potassium oxalate or heparin were used   It seems probable that the loss of platelets in the plasma in these experiments was due to this combination of platelet agglutination and lysis   In an attempt to assess the degree of platelet lysis involved, some of these experiments were repeated and after the platelet counts had been done by the Gram-Thomsen method, 5 hours after the tubes had been set up, the blood was mixed in a mechanical shaker for 5 minutes and platelet counts were then performed on whole blood by the method of Tocantins (10)

TABLE III

Effect of potassium oxalate on the action of sedormid on the platelets of patients who have recovered from sedormid purpura

Patient	Date	Volume of 1 6% potassium oxalate per 1 0 ml blood.	Platelet count on plasma after 5 hours expressed as platelets per c.mm. of whole blood		% loss of free platelets
			Without sedormid	With anti coagulant solution saturated with sedormid	
Case I (N H)	30 10 47 29 1 48	0 125 ml	415,000 616,000	74,000 25,000	82} 96} 89
	21 10 47	0 25 ml	356,000	104,000	71} 79
	30 10 47		444,000	04,000	86}
	30 10 47	0 5 ml	430,000	194,000	55
	11 11 47 13 1 48	10 0 ml	311,000 360,000	316,000 389,000	Nil Nil
	6 1 48 6 1 48 13 1 48 3 2 48	10 0 ml (0 125 ml oxalate diluted to 10 0 ml with saline)	328,000 333,000 406,000 390,000	37,000 58,000 162,000 87,000	89} 83} 60} 78} 78
Case II (M.B)	10 1 48 7 2 48	0 125 ml	365,000 488,000	47,000 26,000	87} 95} 91
	10 1 48 17 1 48	10 0 ml	407,000 418,000	366,000 383,000	10} 8} 9
	17 1 48 7 2 48	10 0 ml (0 125 ml oxalate diluted to 10 0 ml with saline)	377,000 395,000	93,000 47,000	75} 88} 82

TABLE IV

Effect of heparin on the action of sedormid on the platelets of patients who have recovered from sedormid purpura

Patient	Date	Volume and number of units of heparin in saline per 1 0 ml blood	Platelet count on plasma after 5 hours, expressed as platelets per c.mm of whole blood		% loss of free platelets
			Without sedormid	With anti coagulant solution saturated with sedormid	
Case I (N H)	30 10 47	0 05 ml (8 units)	483,000	72,000	85
	17 12 47 21 1 48 2 3 48	0 1 ml (8 units)	500,000 543,000 470,000	140,000 52,000 49 000	72 00 } 84 90
	6 1 48 13 1 48	0 1 ml (80 units)	335,000 364,000	348,000 380 000	Nil Nil
	17 12 47 21 1 48	0 5 ml (8 units)	504,000 510,000	193,000 74,000	62 85 } 74
	6 1 48	10 0 ml (80 units)	374,000	348,000	7
	13 1 48	10 0 ml (160 units)	342,000	377,000	Nil
	10 12 47 21 1 48	10 0 ml (800 units)	284 000 365 000	278,000 371,000	2 Nil
	21 1 48 21 1 48 21 1 48	10 0 ml (8 units)	394,000 435,000 412,000	116,000 104,000 81,000	71 76 } 76 80
Case II (M.B)	24 1 48 27 2 48	0 1 ml (8 units)	307,000 360,000	26,000 20,000	93 94 } 94
	10 1 48	0 1 ml (16 units)	302,000	16,000	95
	17 1 48 24 1 48	0 1 ml (80 units)	409 000 374 000	Platelets numerous but agglu tination too great to permit counting Ditto	
	10 1 48 24 1 48	10 0 ml (800 units)	331,000 360,000	273 000 342,000	18 5 } 12
	24 1 48	10 0 ml (8 units)	290,000	52,000	82
	17 1 48	10 0 ml (16 units)	342,000	64,000	81

referred to above The results of these investigations are shown in Table V Platelet agglutination in these preparations was too slight to cause serious errors in counting, and it seemed that the vigorous mixing combined with the tendency, already commented on, for the agglutinated platelets to free themselves, had caused separation of most of the surviving platelets

When small quantities of citrate, oxalate or heparin were used it was found that although the counts in the sedormid preparations were higher by Tocantins' method than by the Gram-Thomsen method, the counts, as compared with those of blood diluted with the anticoagulant alone, were still considerably lowered This reduction in the platelet count was taken as an indication of the degree of platelet lysis by sedormid in these preparations When the same quantities of these anticoagulants were diluted

TABLE V   •

Comparison of the percentage loss of free platelets with the percentage lysis of platelets by sedormid in blood from two patients who have recovered from sedormid purpura

Patient	Date	Volume of anticoagulant per 1 0 ml blood	% loss of free platelets after 5 hours *	% lysis of platelets after 5 hours †
N.H	29 1 48	0 075 ml. 3 2% sodium citrate	66	38
M B	7 2 48		94	32
N H	29 1 48	0 125 ml 1 6% potassium oxalate	96	76
M B	7 2 48		95	46
N H	2 3 48	8 units heparin in 0 1 ml saline	90	52
M B	27 2 48		94	46
N.H	3 2 48	0 075 ml citrate diluted to 10 0 ml with saline	79	5
M B	7 2 48		82	12
N H	3 2 48	0 125 ml oxalate diluted to 10 0 ml with saline	78	Nil
M B	7 2 48		88	Nil

    * Calculated by deducting the platelet counts in the sedormid preparations, as estimated by the Gram Thomsen method, from the counts in the preparations which did not contain sedormid, and expressing the figure obtained as a percentage of the latter

    † Calculated in the same way as the percentage loss of free platelets , the platelet counts being performed on the same preparations by the method of Tocantins after the blood had been mixed in a mechanical shaker

to 10 0 ml with saline and 1 0 ml of blood was added, it will be remembered that sedormid caused a considerable reduction in the platelet count performed by the Gram-Thomsen method   This, however, must have been due almost entirely to agglutination of platelets with very little lysis, for the counts on whole blood after shaking were not significantly reduced in the sedormid preparations as compared with those which did not contain the drug

This result was surprising and raised the problem whether the sedimentation of the platelets was due to agglutination alone or whether it was due also to some change in the plasma which permitted an abnormally rapid sedimentation of platelets   The answer to this problem was supplied by the observation that if these preparations were allowed to stand again for a further 5 hours after being mixed and the platelet counts repeated by the Gram-Thomsen method, then the platelets remained in suspension in both preparations and in consequence the difference between the counts in the sedormid preparations and those containing the anticoagulant alone, was very greatly reduced (*see* Table VI)   It was therefore concluded that the sedimentation of platelets was due to platelet agglutination and not to any change in the plasma   These experimental results show in addition, that although dilution of blood in the proportion of 10 0 ml of diluent to 1 0 ml of blood does not prevent agglutination it does, to a very large extent, prevent the lysis of platelets by sedormid that occurs in preparations in which the blood is only slightly diluted

The quantitative estimation of platelet lysis by sedormid

Although the use of Tocantins' method of platelet counting provides a rough estimate of platelet lysis it was clearly desirable to have a more accurate technique   The above experiments indicated that the best results

TABLE VI
Table showing that the loss of free platelets caused by sedormid in highly diluted specimens of blood is due mainly to agglutination and not to lysis of platelets or to any change in the plasma causing rapid sedimentation of platelets

Patient	Volume of anticoagulant per 1·0 ml blood	Percentage loss of free platelets by the Gram Thomsen method *	
		Before mixing	After mixing
N H	0 075 ml 3 2% sodium citrate made up to 10 0 ml with saline	69	9
M B		83	12
N H	0 125 ml 1 6% potassium oxalate made up to 10 0 ml with saline	63	21
M B		90	Nil

* For method of calculation *see* Table V   The tubes were left undisturbed for 5 hours and then counted   Each tube was subsequently mixed for 5 minutes and then allowed to stand for a further 5 hours after which the platelets were counted again.

would be obtained when very small amounts of anticoagulant were used and when dilution of the blood was kept to a minimum, even though this entailed the use of very small quantities of sedormid   The following method was found to be the most satisfactory of those which were tried

10 0 ml of blood is transferred in an oiled syringe to a waxed tube containing 80 units of heparin in 0 5 ml of saline   The tube is closed with a waxed cork, rapidly mixed by inversion, and then centrifuged at 2,500 r p m for about 90 seconds   1 0 ml of the supernatant platelet-rich plasma is transferred with a waxed pipette to each of two further waxed tubes, one containing 0 1 ml of saline and the other 0 1 ml of a saturated solution of sedormid in saline   The tubes are closed with waxed corks and their contents mixed by repeated inversion   The tubes are then left on the bench for not less than 5 hours   By this time the platelets in the sedormid preparations will have undergone marked agglutination and will show very gross morphological changes   The majority of the platelets will however still be detectable as " ghost " forms and it has been found very much easier to demonstrate the reduction in the number of platelets in the sedormid containing preparation if the tubes are allowed to stand over night and the platelets are counted on the following day   In order to delay the normal tendency of platelets to undergo disintegration in such preparations 0 1 ml of Liquemin (Roche) is added to each preparation when it has been stood for 5 hours after being set up   Before the platelets are counted the two tubes are repeatedly inverted to ensure adequate mixing   The plasma is then diluted 1 100 with isotonic sodium citrate, mixed for 5 minutes in a mechanical shaker and then transferred to a hæmocytometer chamber The platelets are counted 20 minutes later by which time they will have settled to the bottom of the chamber   The lysis of platelets by sedormid does not appear to be any greater if the preparations are incubated at 37°C and this procedure has the great disadvantage that a small clot sometimes appears in the incubated specimens

Platelet lysis by sedormid was estimated in this way in Cases I and II and the results are given in Table VII   The platelets in the control preparations, which did not contain sedormid, were readily visible in the counting chamber as small highly refractile bodies   In the preparations which contained sedormid there were virtually no normal platelets   There were, however, small ill-defined bodies and larger clumps of material which were probably the remains of incompletely lysed platelets and platelet agglutinates   In the counts which were made on these preparations, anything which could conceivably be called a platelet has been included in the count and for this reason the results given in Table VII are probably an underestimate of the degree of lysis of platelets caused by sedormid in the blood of these patients

TABLE VII

Lysis of platelets by sedormid in heparinised plasma of patients who have recovered from sedormid purpura

Patient	No of determinations for each experiment	Platelet counts per c mm plasma	
		With saline added	With sedormid in saline added
Case I (N H)	3	610,000	310,000
Case II (M B)	3	250,000	42,000

The minimum concentration of sedormid required to cause a reduction in the number of free platelets in the plasma

Thus was estimated in Case II, the platelet counts after 5 hours being performed by the Gram-Thomsen method   The count when 10 0 ml of blood was diluted with 0 75 ml of 3 2% sodium citrate was compared with that obtained when equal volumes of blood were diluted with 0 75 ml of a saturated solution of sedormid in citrate and with 0 75 ml of each of a series of different dilutions of a saturated solution of sedormid in citrate prepared

TABLE VIII

The effect of lowering the concentration of sedormid on its power to reduce clot retraction and the number of free platelets in the blood of a patient (Case II (M.B)) who has recovered from sedormid purpura

Solution used to dilute blood (0 75 ml diluent to 10 0 ml blood)	Platelet count on plasma after 5 hours expressed as platelets per c.mm of whole blood	Clot retraction % when blood clotted with 0 6 ml 1 6% calcium chloride	
		No of expts	
3 2% sodium citrate	370,000	2	53
Saturated solution of sedormid in 3 2% sodium citrate = A	34,000	2	11
Solution A diluted 1 2 with citrate	28,000	—	—
Solution A diluted 1 5 with citrate	97,000	2	39
Solution A diluted 1 10 with citrate	160 000	2	50
Solution A diluted 1 50 with citrate	390,000	—	—
Solution A diluted 1 100 with citrate	380,000	—	—

by adding sodium citrate to the saturated sedormid solution   Several of
these preparations were subsequently clotted with isotonic (1 18%) calcium
chloride and the clot retraction estimated   The results of these experiments
are given in Table VIII from which it will be seen that a significant reduction
in the number of free platelets in the plasma is produced by a dilution of
between 1 10 and 1   50 and a significant reduction in clot retraction by a
dilution between 1 5 and 1 10   A dilution of 1 10 represents a final
concentration of sedormid of 0 00017%   This is approximately a sixth of the
concentration of sedormid (0 001%) that would be found in the body
if a dose of two tablets (0 5 gram) of sedormid was distributed through the
total intra- and extra-cellular body fluid which in a normal adult has a
volume of about 49 litres (7)   It has previously been shown (1) that a reduc-
tion of clot retraction by a solution of sedormid in saline in the same patient
can be produced by a concentration of 0 0006% and it was then
concluded that since the reduction in clot retraction was probably due to an
action of sedormid on the platelets, a deleterious effect on the platelets might
well be produced in these patients by therapeutic doses of the drug   It is
clear from the experiments just reported that this conclusion was entirely
justified

Reduction in number of free platelets in plasma by substances chemically related to sedormid

In an endeavour to discover which components of the sedormid molecule
are principally concerned in causing the phenomena of sedormid hyper-
sensitivity, the action of a series of chemically related substances on the
platelets of sensitised individuals was investigated   This was done by making
saturated solutions of the different substances by boiling them with 3 2%
sodium citrate, allowing the solutions to stand overnight, and then filtering
The solutions were then diluted with 3 2% sodium citrate to a final concentra-
tion approximating to that of a saturated solution of sedormid in 3 2%
sodium citrate (0 025%)   10 0 ml of blood was mixed with 0 75 ml
of each solution in waxed tubes and the platelet counts by the Gram-Thomsen
method performed 5 hours later were compared with that of 10 0 ml of blood
mixed with 0 75 ml of citrate alone   The results of these investigations,
which were performed on Cases I and II, are shown in Table IX, which
also gives the structural formulæ of the various chemicals investigated
Sedormid (allyl-isopropyl-acetyl-carbamide) consists of a molecule of acetyl-
carbamide to the acetyl radicle of which two simple alkyl side chains (allyl
and isopropyl) are attached   The sensitivity of the platelets to the parent
substance, acetyl-carbamide, was first investigated   As this substance
caused no reduction in the number of free platelets in the plasma of either
patient, the effect of the two alkyl side chains when these are attached to a
somewhat dissimilar, though chemically related, substance namely barbituric
acid (malonyl-carbamide) was investigated   This substance (allyl-isopropyl-
malonyl-carbamide) also failed to cause a reduction in the number of free

TABLE IX

Action of sedormid and chemically related substances in reducing the number of free platelets in the plasma of patients who have recovered from sedormid purpura

Chemical investigated	Synonym	Structural formula	Concentration in 3 2% sodium citrate	Number of determinations for each experiment	Platelet count on plasma after 5 hours, expressed as platelets per c mm of whole blood (blood citrated in proportion 10 0 ml to 0 75 ml citrate solution)			
					Date	Case I (N H)	Date	Case II (M B)
3 2% sodium citrate	—	—	—	2	10 4 40 / 18 4 40	500,000 / 500,000	13 4 40 / 24 4 40	300,000 / 410,000
Acetyl carbamide	—	NH—CO—CH₃ / C=O / NH₂	0 03%	2	10 4 40	400,000	24 4 40	410,000
Allyl isopropyl malonyl carbamide	Alurate (Allyl isopropyl barbituric acid)	NH—CO / C=O C< CH(CH₃)₂ , CH₂CH CH₂ / NH—CO	0 01%	2	10 4 40	500,000	24 4 40	410,000
Isopropyl bromo acetyl carbamide	Bromural	NH—CO—CBr< CH(CH₃)₂ , H / C=O / NH₂	0 04%	2	18 4 40	400,000	24 4 40	410,000
Diethyl bromo acetyl carbamide	Adalin	NH—CO—CBr< C₂H₅ , C₂H₅ / C=O / NH₂	0 03%	2	10 4 40	180,000	13 4 40	130,000
Allyl isopropyl acetyl carbamide	Sedormid	NH—CO—CH< CH(CH₃)₂ , CH₂CH CH₂ / C=O / NH₂	0 025%	2	10 4 40	30,000	13 4 40	30,000

platelets in the plasma of either of the patients examined   However, with adalin, in which the two alkyl side chains of the sedormid molecule are replaced by ethyl groups (and, incidentally, the remaining hydrogen atom with bromine) and which has no effect on the platelets of individuals not sensitive to sedormid (*see* Table X), a considerable reduction in the platelet count was obtained in both patients but with bromural, in which the isopropyl side chain of the original sedormid molecule is retained and the other side chain is replaced with an atom of bromine, no effect on the platelets was observed   It is possible that a very slight reaction will sometimes be obtained with this substance because on a former occasion it caused a slight reduction in clot retraction in Case I (N H) (1) although it failed to do so

TABLE X

Effect on the platelet count of the addition of adalin to the blood of patients who were not hypersensitive to sedormid

Patient	Diagnosis	Platelet count on plasma after 5 hours, expressed as platelets per c.mm of whole blood (blood citrated in proportion 10 0 ml. blood to 0 75 ml citrate solution)	
		Citrate	Adalin in citrate
A V	Hæmolytic anæmia 12 days after splenectomy	880,000	880,000
C O	Hypertension	370,000	390,000
S.E	Ulcerative colitis	420,000	430,000
B O	Chorea	320,000	320,000
W H	Gastric ulcer	530,000	550,000
G W	Gastric ulcer	520,000	490,000
E P	Carcinoma of bronchus	390,000	400,000
M C	Hypertensive heart failure	260,000	270,000

2 years later when the present series of experiments was performed *   It was therefore concluded that neither the parent substance, acetyl-carbamide, nor the two side chains when attached to another radicle could precipitate the hypersensitivity reaction, and moreover it appeared that although the presence of one alkyl side chain attached to the actyl radicle of acetyl-carbamide may occasionally be sufficient to produce a very slight effect on the platelets, before any marked effect can be produced, two side chains are required   They need not, however, be identical with those of sedormid

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* The results on this occasion were as follows (each figure is the mean of 4 determinations)
　　2 0 ml. blood + 0 5 ml saline　　　　　　　— Clot retraction = 71%
　　2 0 ml. blood + 0 5 ml. 0 04% bromural in saline — Clot retraction = 73%

Discussion

The cause of thrombocytopenia in sedormid purpura has been the subject of much speculation, reviewed previously (1) Two possible mechanisms have been suggested, namely that the thrombocytopenia is due to an action of sedormid on the megakaryocytes, causing an arrest of platelet production by these cells, or that the drug acts in some way on the platelets in the peripheral blood If it is accepted that platelets are formed by the budding off of portions of the cytoplasm of megakaryocytes then it seems likely that both these mechanisms must be involved for it is difficult to conceive that the process by which the platelet count is reduced could be so specific that it could affect either the platelets or the megakaryocytes alone

Experimental evidence of either of these mechanisms has proved difficult to obtain Examination of the bone marrow has revealed only inconstant and relatively slight changes in the megakaryocytes These changes are not sufficient to prove an action of sedormid on these cells although, as several authors have pointed out, it is possible that they may indicate an inhibition of platelet formation by the megakaryocytes Moeschlin (8) attempted to demonstrate a circulating thrombocytolysin by transfusing blood, on two occasions, from a patient with thrombocytopenic purpura due to sedormid to a normal individual He found no significant change in the platelet count of the recipient on either occasion and therefore concluded that there was no thrombocytolysin in the blood of these patients

It has been shown previously that sedormid reduces clot retraction in the blood of patients who have recovered from sedormid purpura (1) This reduction was later shown to be due to an abnormally rapid lysis of platelets by sedormid during coagulation (2) These findings suggested that the thrombocytopenia in sedormid purpura is probably due to the action of a thrombocytolysin, although the first attempts to demonstrate a lysin by adding sedormid to the blood of such patients in the presence of an excess of sodium citrate had failed because, while sedormid caused agglutination of platelets in the blood of some of these patients, the large amounts of anticoagulant used prevented platelet lysis The experiments reported here, however, show conclusively that sedormid does cause lysis of platelets and, moreover, that a reduction in the number of free platelets in the plasma can be produced in vitro by concentrations of the same order, if not smaller, than would be expected to occur in the blood from the administration of therapeutic doses of the drug Furthermore, it has been shown (2) that the abnormally rapid lysis of platelets that occurs when the blood of these patients is allowed to clot in the presence of sedormid is due to the action of some factor in the plasma and is not due to any peculiarity of the platelets themselves If, as seems probable, these findings are applicable to fluid blood and not only to clotting blood, then it seems clear that, although

sedormid may well have some effect on the megakaryocytes, the thrombo-cytopenia must be due, in large measure, to the presence of a circulating thrombocytolysin which is activated in some way by sedormid

The application of sedormid to the skin of sensitised patients causes a great increase in capillary fragility and the development of hæmorrhages in the area to which the drug is applied (1) This happens in the absence of any significant fall in the platelet count It seems improbable that this capillary lesion could be due to a local destruction of platelets because a supply of fresh platelets would continually be arriving in the blood stream to replace those that had undergone lysis and, moreover, thrombocytopenia by itself is generally considered to be insufficient to cause purpura It also seems improbable that the hæmorrhages could be due to an action of sedormid on the tissue cells as there was no hyperæmia or wheal formation to suggest the release of histamine which usually follows cellular injury The most likely explanation appears to be that sedormid has an effect on the capillaries themselves This concept implies a close antigenic relationship between platelets and the capillary endothelium if it is to be postulated that both can be acted upon by the same lytic factor Bedson has produced evidence of such an antigenic relationship He found (3) that antiplatelet serum, in addition to destroying the platelets of guinea pigs, also damaged the endothelium of the capillaries of these animals and that after the administra-tion of antiplatelet serum " the endothelium appears swollen and œdematous, the cells standing off the vessel wall " In a later paper, the same author (4) stated that it was possible so to grade the dose of antiplatelet serum in rabbits that the platelets could be completely destroyed without vascular damage being caused and consequently, without the occurrence of purpura but that if a larger dose of serum was given then the capillary endothelium was also attacked and hæmorrhages developed

SUMMARY

1 Sedormid causes agglutination and subsequently, lysis of platelets in the blood of patients who have recovered from sedormid purpura This effect can be demonstrated in citrated, oxalated, or heparinised blood It is inhibited by the use of excessive amounts of anticoagulants but is readily demonstrated if the concentration of anticoagulant is kept to a minimum Sedormid has no effect on platelets in the blood of controls

2 Dilution of the blood with ten times its volume of saline almost completely abolishes platelet lysis but does not reduce platelet agglutination

3 The concentration of sedormid required to produce platelet agglutination and lysis is of the same order or less than that which would be expected to occur in the body as a result of the administration of therapeutic doses of the drug

4   The sedormid molecule consists of a molecule of acetyl-carbamide to the acetyl radicle of which two alkyl side chains are attached   Neither the parent substance, acetyl-carbamide, itself, nor the two side chains when attached to another radicle show any effect on the platelets   Substitution of one side chain with an atom of bromine in the sedormid molecule practically abolishes the effect on the platelets but if the two side chains are replaced by two ethyl groups the action on the platelets is preserved to a considerable extent   It therefore appears that before any marked effect on the platelets can be produced two alkyl side chains must be attached to the acetyl radicle of acetyl-carbamide but that they need not be identical with those of sedormid

5   It is concluded that the blood of patients who have recovered from sedormid purpura contains a substance which, in the presence of sedormid, can cause lysis of platelets and that this thrombocytolysin is responsible for the thrombocytopenia that occurs in sedormid purpura   It is suggested that the capillary lesion in sedormid purpura may be due to an action of this same lysin on the capillary endothelial cells

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(a)   (b)
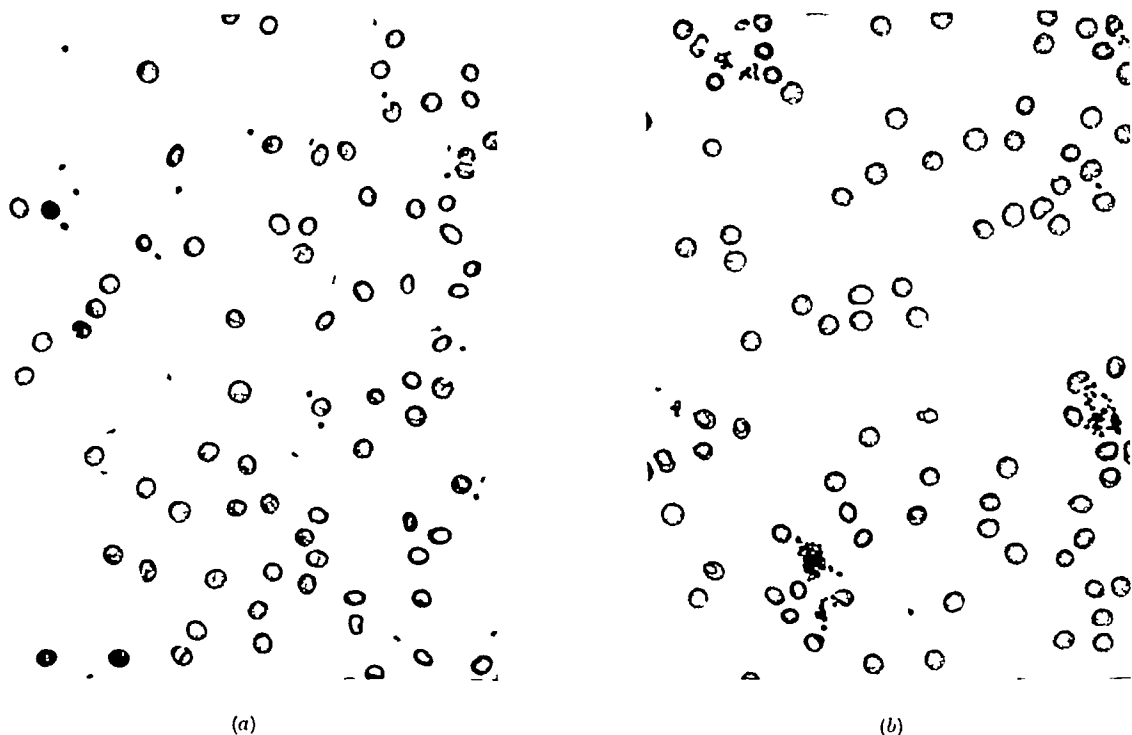
Fig 1   Agglutination of platelets by sedormid in Leishman stained films of citrated blood
from Case II (M B)
(a)   10 0 ml of blood + 0 75 ml of 3 2% sodium citrate   No platelet agglutination
(b)   10 0 ml of blood + 0 75 ml of a saturated solution of sedormid in 3 2% sodium
citrate   Marked platelet agglutination
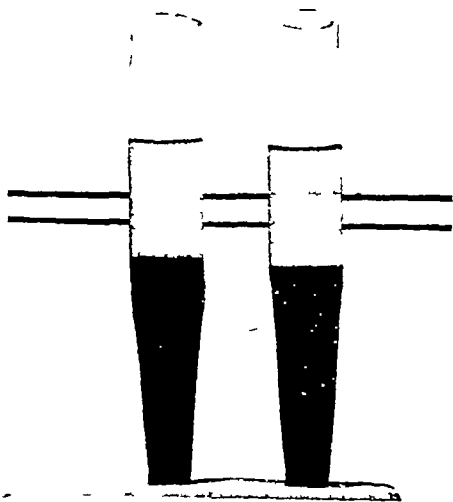
Fig 2   Clearing of plasma as a result of
agglutination and lysis of platelets by sedormid
in blood from Case II (M B)   The blood has
been allowed to stand for 5 hours before being
photographed against a white screen across
which two black lines had been drawn

(a)   10 0 ml of blood + 0 75 ml of 3 2%
sodium citrate   Plasma shows normal opales
cence and is opaque

(b)   10 0 ml of blood + 0 75 ml of a
saturated solution of sedormid in 3 2% sodium
citrate   Plasma is clear and transparent

THE MECHANISM OF PLEURAL AND ASCITIC EFFUSIONS, WITH A SUGGESTED METHOD FOR THE INDIRECT ESTIMATION OF PORTAL VENOUS PRESSURE

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INTRODUCTION

WHEN an effusion of fluid, either ascitic or pleural, is discovered, it can generally be attributed to one, or possibly more than one, of a small number of general mechanisms, namely, venous congestion, either local or systemic, inflammation or carcinomatosis of the serous cavity concerned, hypoproteinæmia or obstruction of lymphatics

But sometimes there is no independent clinical evidence for any of these mechanisms , for example, ascites may be the only manifestation of portal obstruction   In other instances, the diagnosis being known, the mechanism of the effusion remains in doubt   For example, a pleural effusion in a patient with tuberculous polyserositis may be due either to venous congestion resulting from pericardial involvement, or to tuberculous pleurisy (1) , or again, ascites may be caused in a patient with carcinoma by carcinomatosis of the peritoneum or by pressure of an enlarged lymph node on the portal vein (23)

Nor is it only in individual cases that the mechanism of an effusion is sometimes uncertain , thus it is still debated whether the ascites which often accompanies cirrhosis of the liver results mainly from portal obstruction, hypoproteinæmia, or from water and salt retention (13, 28)

The general causes of effusions listed above may all be regarded as disturbances of the equilibrium of hydrostatic and colloid osmotic forces which was shown by Starling (31) to exist between intravascular and tissue fluid, and which might be expected to exist also between the blood and pleural or ascitic fluid   This paper describes an attempt to throw light on the mechanisms of effusions by measurement of the component forces of such an equilibrium

Three of the four desired measurements, namely the effusion fluid pressure, and the colloidal osmotic pressures of plasma and effusion fluid can readily be made   There remains the pressure in the capillaries with which the effusion is in equilibrium, which cannot be determined directly Instead, the systemic venous pressure has been measured, as being a quantity which will, with certain exceptions, bear an approximately quantitative relation to capillary pressure (17)   Consideration of the results of such measurements leads to the conclusions that the factors causing the maintenance of serous effusions are similar to those identified by Starling as responsible for tissue fluid formation and absorption, and that portal obstruction is the predominant cause of ascites associated with cirrhosis of the liver   Analysis of the fluid protein levels reveals their dependence on two factors, namely the plasma albumin level, and on the nature of the disease causing the effusion , considerable modification of the traditional division of effusions into exudates and transudates is found to be necessary Finally, the significance of the low plasma protein levels found in patients with effusions is discussed

METHODS

Determination of venous pressure—effusion fluid pressure difference

Venous and effusion fluid pressure were determined simultaneously, the manometer tubes being fixed vertically on either side of a centimeter scale The sternal angle was chosen as the anatomical point of reference, and its position on the scale determined with a spirit level, the pressures referred to this point being obtained by subtraction

The venous pressure was determined by connecting a citrate manometer to an antecubital vein , care was taken that the arm was not in contact with the chest, as such a position produced an erroneously high reading, and that it lay below the sternal angle   In 25 patients in whom there was no evidence of congestive failure, the venous pressures so recorded varied from 5 5 cm above to 4 0 cm below the sternal angle, with a mean of + 0 075 cm   In three patients (Cases 2, 31 and 36) it was not possible to make a direct measurement of the venous pressure, and the level of filling and pulsation of the neck veins was used instead (18)

The effusion pressure was measured by connecting a simple manometer tube to the instrument used for paracentesis, and allowing the fluid to reach a level in it   This level varied with respiration to an extent depending on the size of the cannula or needle used , the mean position between the extremes of respiratory variation was taken as the effusion pressure   This pressure was also referred to the sternal angle as a purely arbitrary point of reference, since it was primarily desired to compare venous and effusion pressures rather than to determine their absolute values   The choice of an antomical point of reference more or less remote from the effusion, while

it makes the pressure recorded independent of the site of paracentesis, has the consequence that the pressure will be affected by alterations of posture The pleural effusions were all tapped with the patient sitting upright and leaning slightly forwards , the abdominal paracenteses were performed with the patient lying on his back with his head and shoulders raised on three or four pillows, no attempt being made to standardize more exactly the angle at which the patient lay

In a patient of average size, an alteration of posture from sitting upright to lying flat alters the height of the sternal angle relative to the centre of an ascitic effusion by about 30 cm , it might be anticipated, however, that the ascitic pressure would not increase by this theoretical amount when the patient lies down, because the liver and other viscera would no longer be compressing the effusion to the same extent   In order to estimate the error in the determination of venous pressure-effusion pressure difference that was introduced by variations in posture from case to case, measurements of venous and effusion fluid pressure in different postures were made in three patients with ascites, one with a pleural effusion and one with a large ovarian cyst (the latter not being otherwise described in this paper)   In none of the 5 patients did alteration in posture produce a consistent effect on venous pressure   In the three patients with ascites, the change from

TABLE I

Shows the venous pressure effusion fluid pressure difference (cm of water , venous pressure above effusion pressure taken as positive) in different postures in three patients with ascites, one with a pleural effusion, and one with an ovarian cyst

	Ascites			Pleural effusion	Ovarian cyst
Upright	—1 0	—3 5	+9 5	+5 5	—7 0
Semi recumbent	—1 0	—4 0	+4 5	+7 5	—8 5
Flat	+1 3	—5 0	+1 5	—	—11 5

upright to flat caused a rise in ascitic pressure relative to the sternal angle varying from 1 0 to 8 0 cm of water   In Table I are shown the venous-ascitic pressure differences in each patient in the different postures   The standard deviation of the individual estimations about the mean for each patient was 1 96 cm , making the possible error in venous-fluid pressure differences ± 4 0 cm over the range of postures described, a greater range than that encountered during the main part of the investigation   Only a small part of the horizontal scatter of the points in Figs 2 and 4 can therefore be ascribed to variations in the position of the patients

Protein estimation

Blood for protein estimation was taken into potassium oxalate, usually from the needle used for venous pressure determination, the first few ml being discarded owing to possible admixture with citrate   Except for the last 9 observations, in which a standard concentration of 0 08 mg of pot oxalate per ml. blood was used, the quantity of oxalate used was not measured This omission has introduced a variable systematic error into the estimation of the plasma proteins because it is known (25) that the addition of pot oxalate to blood dilutes the plasma proteins by withdrawing protein free fluid from the red cells   To estimate the magnitude of the error so introduced, aliquots from two samples of heparinized blood were mixed with different quantities of oxalate and centrifuged after a standard period   The magnitude of the dilution produced is shown in Table II

TABLE II

Effect of potassium oxalate on plasma protein

Concentration of potassium oxalate mg per ml	0	1	2	5	20
Plasma A. Total protein g per 100 ml	7 40	7 32	—	6 78	6 19
Plasma B Total protein g per 100 ml	7 60	—	7 10	6 73	—
Hæmolysis	0	0	±	+	++

With concentrations of potassium oxalate in blood exceeding 5 mg per ml , visible hæmolysis was produced, and this was not present in plasma used in the determinations recorded in this paper   The concentration of oxalate used in most observations probably lay between 2 and 3 mgm /ml , which causes dilution of the order of 5%   This is in agreement with the findings of Peters, Eisenman and Bulger (25), who showed that 0 2% of potassium oxalate (2 mg per ml) produced a mean dilution of 5 3% in three samples of plasma

In one experiment in which two pairs of samples of heparinized blood were left in contact with 2 and 5 mg/ml of oxalate for 5 minutes and 2 hours respectively before centrifuging, the dilutions produced by 2 mg per ml were 6 6 and 6 6% respectively, and by 5 mg per ml 11 4 and 10 9%   There was, therefore, no evidence that, within the limits of the delay sometimes incurred during this investigation, the time elapsing before the blood was centrifuged affected the plasma protein concentration.   No correction for dilution by oxalate has been applied to the plasma protein levels herein recorded

For estimation of protein in effusion fluid, a sample was taken into oxalate immediately before drainage of the fluid was begun

In all the observations on ascites, and in 11 of the 17 on pleural effusions, the albumin and globulin content of plasma and fluid were determined by the micro Kjeldahl method, globulin being separated by the method of Howe (14)   In the first 6 observations on pleural effusions (Cases 3, 5, 6, 7, 10, 12) the proteins were determined colorimetrically, using Folin and Ciocalteu's reagent (10)   The digestion mixture used was 1 ml of concentrated sulphuric acid, 0 5–1 0 g of potassium sulphate, 10–15 mg of copper sulphate and about 1 mg of selenium   For the tungstic acid filtrates, 2 ml of sulphuric acid were used, and potassium sulphate was not added to the sodium sulphate filtrate   The mixtures were digested on gas burners until clearing had taken place, and acid was condensing from the neck of the flask, the whole process being complete in 15–20 minutes   To determine whether digestion was complete in such a period, two samples of plasma, one of ascitic fluid, and one of sodium sulphate filtrate from plasma were digested for varying times   The results are shown in Table III   There is no evidence from these figures that digestion for as long as 7 hours significantly affects the nitrogen estimation, as compared with digestion of minimal duration   In two samples of plasma, the total protein was determined both by the micro Kjeldahl and by the gravimetric method (11)   The results are as follows —

	Micro Kjeldahl	Gravimetric
Sample A g per 100 ml	7 03	6 91
Sample B g per 100 ml.	7 11	7 04

From the protein levels so obtained, the colloid osmotic pressures of plasma and fluid were calculated and expressed as cm of water

The imperfect agreement between different authors (8, 9, 34, 36) as to the relation between albumin and globulin concentrations and colloid osmotic pressure is reviewed by Higgins, Kelsall, O'Brien, Stewart and Witts (13)

The three formulæ of these various authors are derived from observations of the colloid osmotic pressure of samples of plasma whose albumin and globulin content had been determined by methods similar to those used in this investigation It is here desired, however, to use the formula also for the estimation of colloid osmotic pressure from the generally much lower concentrations of protein found in effusion fluids, and such an extension of the original purpose of these formulæ may lead to erroneous results ; since it has to be assumed that they are applicable to a range of protein concentrations lower than that from which they are derived, and also that the osmotic pressures per gramme of the protein fractions in effusion fluid are identical with those of plasma proteins A formula is here needed which relates most accurately colloid osmotic pressure and protein concentration over a range which includes low values

TABLE III

Effect of digestion time on estimation of total protein (g per 100 ml)

Time of digestion	Plasma A	Plasma B	Ascitic fluid	22% sod sulphate filtrate
Until clear	6 47	7 25	3 84	4 17
1 hour	—	—	3 90	—
2 hours	—	—	3 76	—
4 hours	6 40	7 28	—	4 25
7 hours	—	—	3 90	—

The equation of Wies and Peters (36) seemed unsuitable in that it gave negative osmotic pressures for effusion fluids with low protein content Both Wies and Peters, and Wells, Youmans and Miller (34) seem to be agreed that osmotic pressures estimated by Govaerts' (8) formula are too high, and at low A/G ratios, Govaerts' own formula seems to give a higher estimate than his observations would lead one to expect (9) Thus in his scatter diagram showing the relation between osmotic pressure per gramme protein, and A/G ratio, 6/7 of the points representing A/G ratios less than 0 75 lie below his theoretical curve Because it seemed to satisfy the above-mentioned conditions most adequately, the formula of Wells, Youmans and Miller (34), namely

$$OP = (0\ 59A + 2\ 14)C$$

(where OP is the osmotic pressure in cm of water, A, albumin, and C, total protein in g per 100 ml) has been used in this investigation

Patients studied

Observations were made on seventeen patients with pleural effusions due to congestive failure (5) constrictive pericarditis (2) carcinomatosis (5) and presumed tuberculosis (5)

The evidence for the diagnosis in each case was as follows —

Heart failure All had venous congestion, dependent œdema and enlargement of the heart The heart disease was caused by rheumatic carditis with mitral stenosis and auricular fibrillation (Case 1), aortic regurgitation due to bicuspid aortic valve discovered at post mortem (Case 2), myocardial infarction (Cases 3 and 4), and hypertension (Case 5)

Constrictive pericarditis Constrictive pericarditis was confirmed at operation in Case 6 and post mortem in Case 7 Peritoneal tubercles had been seen by peritoneoscopy in Case 6, but thoracoscopy revealed no evidence of tuberculous pleurisy At post mortem examination of Case 7, all the serous cavities showed tuberculous inflammation In neither patient were tubercle bacilli recovered from effusion fluid

Carcinomatosis The five neoplastic effusions included two due to carcinoma of the bronchus (Cases 10 and 12), one secondary to carcinoma of the cervix (Case 8) and one due to reticulosis (Case 11) These were confirmed post mortem Case 9 was a male patient aged 67 who had had cough and pain in the chest for 6 months A bloodstained right sided effusion was repeatedly tapped, and X ray of his chest showed an ill defined opacity in the retro hilar region He died at home and no post mortem examination was made

Tuberculosis Case 16 was a patient with polyserositis, having also ascites due to tuberculous peritonitis confirmed at operation In the others, no certain evidence of tuberculosis was forthcoming Cases 13 and 17, aged 42 and 22, had had symptoms for 6 and 2 weeks respectively, and their effusions absorbed in 2 and 4 weeks Case 15, aged 27, had had cough and night sweats for a month In addition to his effusion, X ray revealed a parenchymal inflammatory lesion of the right upper lobe His effusion did not absorb during his stay of 6 weeks in this hospital Case 14, aged 66, was at first believed to have a neoplastic effusion, but his condition improved so much that this was thought to be excluded and an infraclavicular infiltration was regarded as tuberculous

Twenty five observations were made on 24 patients with ascites , two observations on Case 18, having been made 11 months apart, are both included The ascites was caused by congestive failure (5), constrictive pericarditis (3), neoplasm (5), nephrosis (1), tuberculosis (1), and cirrhosis (9)

Heart failure All had venous congestion and dependent œdema, although this was slight in Cases 18 and 22

The heart disease was due to rheumatism with mitral stenosis and aortic regurgitation (Cases 18 and 19), rheumatism with mitral and tricuspid stenosis confirmed at post mortem (Case 22), hypertension (Case 20) and doubtful causes, ? beri beri (Case 21)

Constrictive pericarditis Cases 23, 24, and 25 were male patients in whom fibrous con strictive pericarditis of undeterminable ætiology was found at operation

Carcinomatosis Case 11, in whom a reticulosis of uncertain type was found at post mortem, had extensive neoplastic infiltration of the peritoneum Post mortem examination of Cases 28 and 30 showed carcinomatosis of the peritoneum, the primary being in the gall bladder and stomach respectively In Case 26, although there was extensive carcinomatosis of the peritoneum, no primary site could be identified post mortem Case 27, still alive at the time of writing, is a woman aged 65, whose abdomen has been swollen for 5 months , her ascites has been repeatedly tapped, and she has lost weight and strength No primary lesion has been discovered, and the neoplastic nature of this effusion, while highly probable, cannot be regarded as established beyond all doubt

Tuberculosis Case 16, as already mentioned, had tuberculous peritonitis verified by operation

Nephrosis Case 29, a male aged 16, had had swelling of his legs, abdomen and face, for four months, and had heavy albuminuria The amount of ascites was small

Cirrhosis 5 patients in this group had multilobular cirrhosis, verified at operation in Cases 31, 34 and 37, and post mortem in Cases 32 and 36 In Cases 35 and 39, the diagnosis rested on clinical grounds Case 35, a woman aged 60, with an excessive consumption of alcohol had had 3 hæmatemeses during 8 years and ascites for 18 months, which required tapping every fortnight Case 39, a man aged 41, had had jaundice, 5 years ago, and a hæmatemesis 1 year ago, at which time his liver and spleen were found to be palpable A progressive increase in his serum thymol turbidity was observed. His ascites had been present for 2 months Case 33 had ascites associated with jaundice due to subacute hepatitis of 18 months duration, verified by hepatic biopsy Her liver and spleen were palpable

Case 38, had jaundice and ascites A liver biopsy taken at operation showed marked biliary cirrhosis She also had gallstones , histological examination of the bladder gall revealed suspected malignancy, but there was no invasion or metastasis

RESULTS

Pleural effusions

The venous and pleural pressures, and the concentrations of albumin and globulin in plasma and pleural fluid in each patient are shown in Fig 1

In the patients with congestive failure and constrictive pericarditis the venous pressures were raised, varying from 25 5 to 8 0 cm above the sternal angle

One patient (Case 8) with carcinomatosis, who was gravely ill, dying 5 days after the observation was made, had congested neck veins, and an antecubital venous pressure of + 10 0 cm   In the other patients with carcinomatosis and tuberculosis, venous pressures were normal, ranging from + 5 5 cm to — 2 5 cm   The pleural pressure was greater in those with
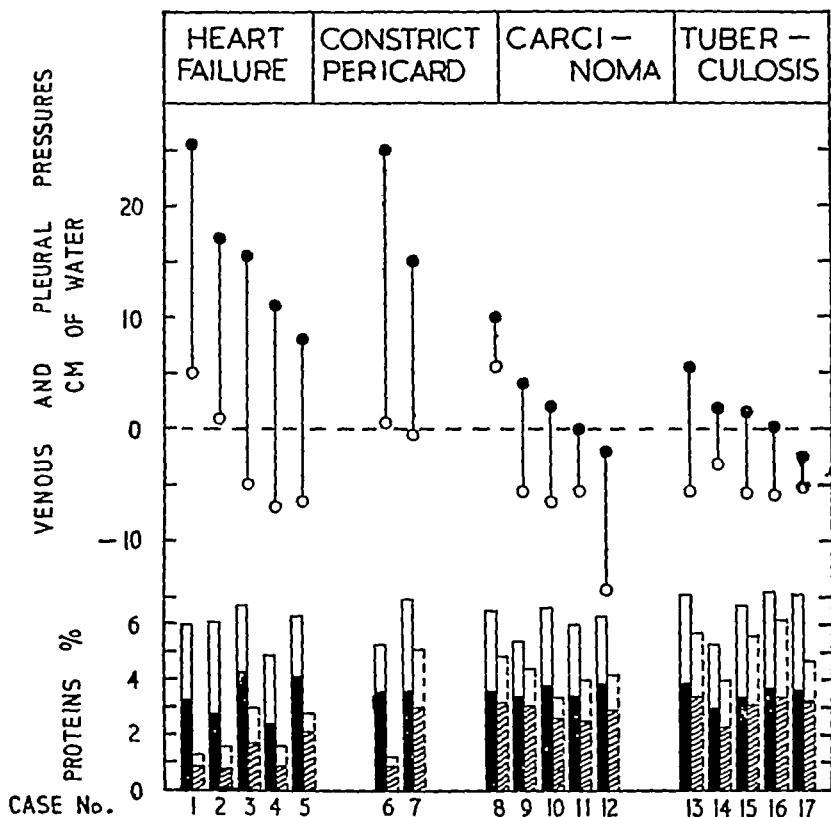
Fig 1   Shows the venous pressure (black dots) and pleural pressure (clear circles) in each of 17 patients with pleural effusions due to the causes shown   The broken line represents the level of the sternal angle   The patients in each group are arranged in order of descending venous pressure   Below are shown the plasma protein level (left-hand rectangle formed by continuous line) and fluid protein level (right hand rectangle formed by dotted line)   The plasma albumin fraction is represented by a black rectangle, and the fluid albumin fraction by a shaded rectangle

increased venous pressure than in those without, and was lower than the venous pressure in all patients   The mean pleural pressure for the congestive failure and constrictive pericarditis groups combined was — 1 8 cm , for the carcinomatosis group, excluding Case 8, — 8 0 cm , and for the tuberculous group — 5 0 cm   there is much overlapping of individual values in different groups

The difference between the venous and pleural pressures was greater in patients with venous congestion than in those without, the means being 18 3 cm for congestive failure, 20 0 cm for constrictive pericarditis, 8 0 cm for carcinomatosis and 6 4 cm for tuberculosis

The albumin and globulin content of plasma and of pleural fluid in each patient is also shown in Fig 1   The plasma protein levels are comparable in all groups   The fluid proteins were higher in carcinomatosis and tuberculosis than in congestive failure   The two patients with constrictive pericarditis had widely different fluid proteins
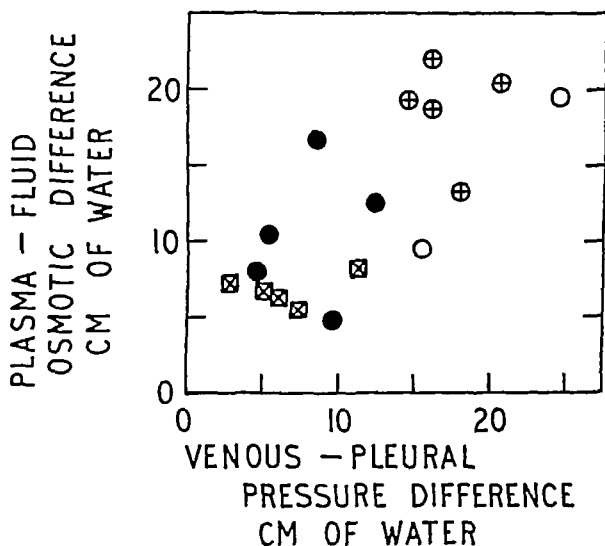
Fig 2   Ordinate   plasma colloid osmotic pressure minus pleural fluid colloid osmotic pressure (cm of water)   Abscissae   venous pressure — pleural pressure (cm of water)
⊕ — Heart failure   ● — Carcinomatosis
O — Constrictive pericarditis.   ☒ — Tuberculosis

General inspection of Fig 1, suggests that it may be possible to correlate the difference between venous and pleural pressure with the difference between the colloid osmotic pressures of plasma and pleural fluid

These quantities are plotted in Fig 2, from which a high degree of correlation (r = 0 83, P < 0 001) is apparent   The relation is an approximately linear one, passing near the origin   Pleural effusions due to carcinoma or tuberculosis provide the lower values for the two quantities plotted, and those due to congestive failure or constrictive pericarditis the higher

Ascites

The venous and ascitic pressures, and plasma and fluid proteins are shown in Fig 3

One patient with carcinoma was very ill and her venous pressure was + 7 0 cm In the remainder, normal venous pressures were found except in the congestive failure and constrictive pericarditis groups

Ascitic pressure showed little variation between the groups except that in cirrhosis it tended to be high
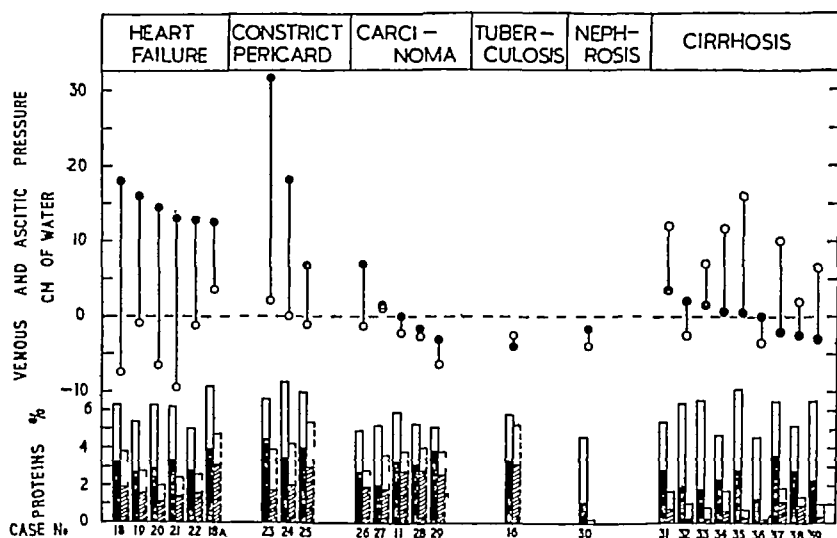
Fig 3   Shows the venous and ascitic pressure, and plasma and ascitic fluid proteins in each of 24 patients with ascites due to the causes shown   Notation as in Fig 1

In heart failure and in constrictive pericarditis the ascitic pressure was well below the venous pressure   In carcinoma and in the case of nephrosis, although ascitic pressure was still below the venous pressure, the difference was very small   In the one patient with tuberculous peritonitis the ascitic pressure was slightly above the venous pressure   In cirrhosis, with two exceptions, the ascitic pressure exceeded the venous, in some instances by a substantial amount

The protein contents of plasma and ascitic fluid from each patient are also shown in Fig 3   The plasma albumin levels were low in cirrhosis, and in the one patient with nephrosis   In the remainder, the plasma albumin levels were similar in all groups   The ascitic fluid protein levels were higher in tuberculosis and carcinomatosis than in congestive failure, although the difference is rather less conspicuous than that in pleural effusions   In cirrhosis and nephrosis the fluid protein was extremely low

In Fig 4, the difference between venous and ascitic pressure is plotted against the difference between the colloid osmotic pressures of plasma and ascitic fluid   Excluding those representing patients with cirrhosis, the points are significantly correlated ($r = 0.86$) and obey an approximately linear relation with a slope considerably smaller than that in Fig 2, crossing the osmotic pressure difference axis at $+ 5.0$ cm   As in pleural effusion, patients with venous congestion provide the higher values represented in this diagram, and those with carcinomatosis, tuberculosis or hypoproteinæmia the lower
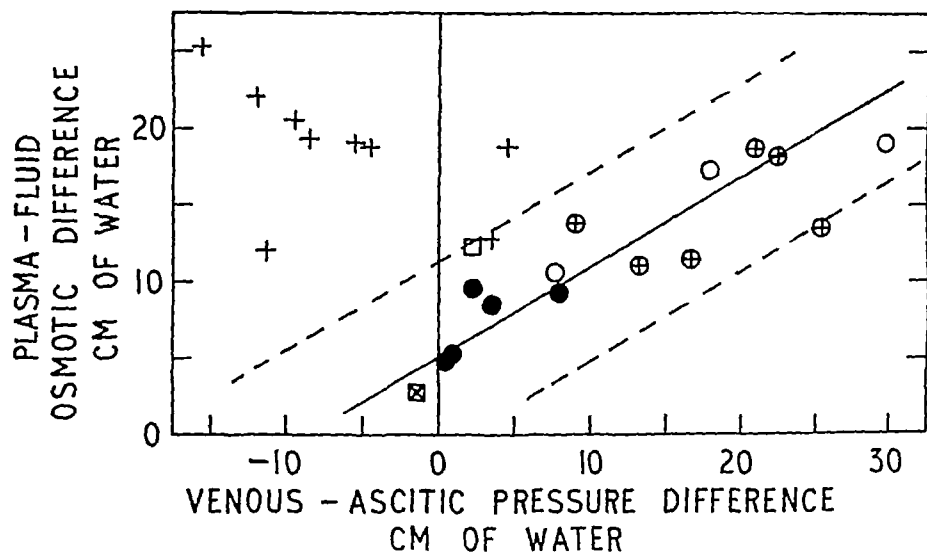
Fig 4   Ordinate   plasma colloid osmotic pressure — ascitic fluid colloid osmotic pressure (cm of water)   Abscissæ   venous pressure minus ascitic pressure (Cm of water)
　　　　□ — Nephrosis   ╋ — Cirrhosis of liver   Other symbols as in Fig 2
The continuous line is the regression line of venous ascitic pressure difference on colloid osmotic pressure difference, excluding patients with cirrhosis   The dotted lines show the limits of twice the standard deviation of the points about the regression line in a direction parallel to the abscissæ

With one borderline exception, the points representing patients with cirrhosis lie significantly to the left of the remainder   All but two, having an ascitic pressure greater than the venous pressure, take negative values on the abscissæ, while the osmotic pressure differences are relatively high, mainly owing to the extremely low protein content of the ascitic fluid in these patients

Pressure changes during paracentesis

In seven patients with ascites, the ascitic pressure was measured at intervals during the removal of fluid, the total volumes removed varying from 600 ml to 6 litres   The pressure always fell during paracentesis, and for volumes up to 4 litres the relation between the fall in pressure and the volume of fluid removed was linear, the slope of the relation providing an estimate of the elasticity of the abdominal wall and other structures containing the ascites

The fall in pressure per litre of fluid removed varied from 2 0 to 7 2 cm of water   The three highest values were found in one patient with constrictive pericarditis and tuberculous peritonitis, and two with carcinomatosis of the peritoneum   Two of the other patients had congestive failure, one had constrictive pericarditis, and one cirrhosis of the liver

TABLE IV

Case 18   Rate of fall of ascitic pressure during paracentesis

Date of observation	Rate of fall cm of water/litre of fluid removed.
29 11 46	3 2
20 12 46	2 8
5 1 48	2 0

In Case 18 a progressive fall in the elasticity of the abdominal wall was observed, the figures being shown in Table IV   This provides some evidence that long-continued ascites tends to increase its volume by progressive distension of the abdominal wall , in the other patients, however, there was no obvious correlation of the elasticity of the abdominal wall with the age of the effusion or the frequency with which it had to be tapped

During removal of pleural fluid, the fall in pressure was not linearly related to the volume of fluid removed, but the pressure fell slowly at first, and more rapidly as the last few ounces were taken off   The overall rates of fall in pressure varied from 5 to 30 cm of water per litre

DISCUSSION

Starling's equilibrium as applied to effusions

The concept from which this investigation derives, namely that an effusion of fluid is always in hydrostatic and osmotic equilibrium with circulating blood, can only be partially valid, because any disturbance of the equilibrium will initiate formation or absorption of fluid, which is a process requiring time for its accomplishment

Thus in one patient with ascites due to neoplasm, after removal of 9 pints of fluid the ascitic pressure fell by 5 cm of water, and took 6 days to regain its previous level   Similarly, Mankin and Lowell (21) found that, following paracentesis, the ascitic fluid volume as measured by the dye dilution method increased rapidly for three days, and after that more slowly

In the present investigation, 3 patients with pleural effusion and 9 with ascites had had fluid removed before the paracentesis at which the pressure measurements were made, but the bulk of the fluid had reaccumulated

In these circumstances both Mankin and Lowell (21) and the author have found that further changes in pressure are small   Pressure readings made in such patients are therefore presumed to be comparable with those made on an effusion being tapped for the first time

Disequilibrium may also arise from sudden alterations in the component forces   Substantial alteration in the colloid osmotic pressure of plasma or of fluid can only be gradual, but the same does not apply to the hydrostatic forces   The changes in pressure which have been shown to result from alteration in posture make it necessary to suppose that, in so far as a single position is never maintained for days at a time, effusions can never come completely to equilibrium with the blood   In this investigation all the patients with the exception of Case 35 had been in bed for at least 24 hours before the observations were made

The venous pressure of patients with heart failure is liable to sudden and large variations, particularly tending to fall rapidly when treatment is begun   The venous pressure at the time of paracentesis is therefore liable to differ from that prevailing at the time when the effusion was formed

Apart from such practical considerations, the simple conditions envisaged by Starling for tissue fluid cannot, for anatomical reasons, obtain for pleural or ascitic effusions   Each of these cavities is drained by two sets of capillaries, systemic for the parietal layer, and pulmonary (30) and portal respectively for the visceral, and there is every likelihood that the pressures in the two systems sometimes differ, in which case, pleural or peritoneal capillary pressure becomes an abstraction

For these reasons, and because of the use of venous instead of capillary pressure, it cannot be expected that the observations recorded in this paper can be exactly interpreted in terms of Starling's theory   If, however, cirrhosis of the liver is excluded, because in this condition a new factor of portal obstruction intervenes, then the findings are clearly in general agreement with Starling's theory in that a proportionality has been shown to exist between the colloid osmotic and hydrostatic forces identified by Starling (31)

Portal obstruction and ascites

Fig 4 shows that a relation exists between the difference between venous and ascitic pressure, and the difference between the colloid osmotic pressures of plasma and of ascitic fluid, except in ascites due to cirrhosis or hepatitis

The direction of the discrepancy would be explicable by an increase in portal venous pressure above systemic , a fixed relation no longer existing between the two, the venous-ascitic pressure difference would be under-estimated by an amount depending on the degree of portal obstruction

One is tempted to suppose that, were portal venous pressure substituted for systemic, the discrepancy due to portal obstruction would disappear, and ascites due to this cause would obey the same relation as that due to other causes

If this assumption is made, the increase in portal venous pressure above systemic is represented by the difference between the observed "hydrostatic pressure difference" (venous pressure-ascitic pressure) and the value which it should have if portal obstruction were absent

This difference can be estimated graphically from Fig 4, being the horizontal distance from any of the points to the continuous line (The line is the regression line taking osmotic pressure difference as independent variate, since it is desired to estimate the most probable value of venous pressure-ascitic pressure for a given osmotic pressure difference)

The same difference can be obtained numerically from the following formula derived from data in the graph

$$PVP = AP - VP + 1\ 7\ (OPD - 5)$$

where PVP is the excess of portal venous pressure above systemic, AP is the ascitic pressure, VP the antecubital venous pressure, and OPD the difference between the colloid osmotic pressures of plasma and of ascitic fluid, all measured in cm of water

The broken lines in Fig 4 represent the limits of twice the standard deviation of the points about the regression line in a horizontal direction, excluding points representing patients with cirrhosis, and give an estimate of the limits of normal of $\pm$ 11 cm

The increase in portal pressure measured in this way in 9 patients with cirrhosis or hepatitis varied from 10 to 50 5 cm, with a mean of 30 1 cm The results obtained by other authors by direct measurement at operation of portal venous pressure have been expressed as cm above the portal vein, which has a rather uncertain relation to the sternal angle The portal pressures here estimated, being the difference between portal and systemic venous pressure, are most easily compared with results of simultaneous measurement of portal and limb vein pressures Whipple (35) measured the pressure in the splenic vein simultaneously with that in an arm vein in 5 patients with Laennec's cirrhosis The difference varied from 17 to 33 cm of water, with a mean of 26 6 cm Bellis (4) found that the difference in pressure in the portal vein and in an ankle vein in one patient with portal cirrhosis and ascites was 32 cm Blakemore (5) found that the mean portal venous pressure in 6 patients with Banti's syndrome was 33 2 cm and in 7 patients with portal cirrhosis 29 4 cm the pressures were referred to the portal vein, and he does not record systemic venous pressure measurements

The mean excess of portal pressure estimated in the present series of 9 patients with cirrhosis (30 1 cm) therefore compared very closely with direct measurements made on similar patients

Hæmatemesis, in a patient with cirrhosis of the liver, being a manifestation of dilated collateral veins, may be expected to occur in those in whom portal hypertension is most severe Of the 9 patients in the present series, three had suffered from hæmatemesis and these three had the three highest estimated portal pressures Their mean portal pressure was 42 6 cm , and that for the six who had not had hæmatemesis 23 8 cm the difference is significant (t = 3 5, P = 0 01)

Although, therefore, it has not been possible to compare estimations of this kind with direct readings of portal venous pressure, it seems justified to say that the estimates are of the right order, and that the method is capable of distinguishing mild from severe portal obstruction

The method may prove to have practical application in helping to distinguish those patients with gross portal hypertension who may be most suitable for a porta-caval anastomosis, a distinction which can at present be made only during operation by direct cannulation of the portal vein

In suggesting this method of estimating the portal venous pressure, it must be emphasized that a small error in osmotic pressure difference will have a great effect on the estimate of portal pressure, owing to the slope of the regression line in Fig 4 , consequently protein estimations must be accurate

These observations stress the importance of portal hypertension in the causation of ascites in cirrhosis of the liver The association of ascites with hypoproteinæmia in patients with cirrhosis has been emphasized by many authors (22, 33, 27, 13), while Ralli, Robson, Clarke and Hoagland (28) suggest that such ascites is largely a manifestation of water retention caused by failure on the part of the liver to destroy antidiuretic substances

If the above method of estimating the degree of portal obstruction be accepted, it is possible to judge the relative importance of hypoproteinæmia and portal obstruction The mean plasma osmotic pressure for the patients with cirrhosis was about 12 cm below normal, as compared with a mean increase of portal pressure of 30 1 cm In some patients one factor preponderated to the virtual exclusion of the other Thus in Case 37 the increase in portal pressure was only 10 cm giving a value which is just within the limits of normal, while his plasma osmotic pressure was 13 4 cm , only a little greater than that of the patient with nephrosis Case 36 on the other hand, had an increase in portal pressure of 50 5 cm , and a plasma osmotic pressure of 26 9 cm

There is no evidence from the present findings that portal obstruction was at all responsible for ascites associated with chronic congestive heart failure

Two patients showed a clinical picture sometimes ascribed to portal obstruction due to cardiac cirrhosis Case 18 had had congestive failure for 12 years and ascites for 14 months, and Case 22 failure for 4 years and

ascites for 1 year   Both these patients had relatively little œdema of the legs and sacrum, and were only admitted to hospital at intervals for paracentesis

Case 22 died of a pulmonary embolus, and had severe fibrosis of the liver which is described by Professor W D Newcomb as follows —

" The liver shows long standing venous congestion with considerable destruction of whole liver lobules in some parts and only the centres of the lobules in others   There is much fibrosis especially under the capsule with considerable proliferation of small bile ducts   Although some of the portal veins are large, in most of the lobules there is no evidence of hypertrophy of the walls of the portal veins "

Although cardiac cirrhosis might be suspected in Case 18 and was shown to be present in Case 22, there is no evidence from the ascitic pressures that the changes produced in the liver are such as to cause portal obstruction This is in keeping with the finding of Katzin, Waller and Blumgart (15) that ascites was not conspicuously commoner in patients with heart failure who had fibrosis of the liver than in those who did not   In only 6 of the 15 patients in their series whose ascites required paracentesis was fibrosis of the liver demonstrable

The protein content of effusion fluids

The traditional division of effusion fluids into transudates containing little protein, and exudates containing a greater amount is widely held to account for the low protein content of the fluids found in cirrhosis and nephrosis on the one hand, and for the high level in tuberculosis on the other (19, 20)   The intermediate protein levels found in the effusions of congestive failure fit less easily into this framework, and it is usual to account for them by postulating increased capillary permeability due to anoxia or dilatation (7) or by concentration of low protein fluid by diuretic treatment (16)

In an attempt to define the factors responsible for the protein levels of the effusion fluids of the present series it has been a guiding principle to treat albumin and globulin separately, because these proteins, differing as they do in molecular weight (6) must be expected to behave differently in their tendency to enter effusion fluids   For this reason, confusion may be minimised by avoiding as far as possible such quantities as the A/G ratio or the proportionate composition of the total protein content, and considering only absolute levels of albumin and globulin   Even this separation is only a first approximation   For example Wolfson, Cohn, Calvary and Thomas (37) point out that the filtrate from 22% sodium sulphate contains both true albumin and α-globulin, as may be detected by electrophoresis, and by more complex salting-out methods

It is here proposed to analyse the protein content of effusion fluids with respect to two factors, namely the plasma protein level, and the nature of the disease causing the effusion

(1) *Plasma protein level*

   In Figs 5 and 6, plasma albumin and globulin levels are plotted against the corresponding fluid albumin and globulin levels, pleural effusion and ascites being kept separate   Briefly, there is an association of the albumin levels of plasma and effusion fluid, but not of the globulin levels except in tuberculous pleurisy

   The lower part of Fig 5, which relates plasma and fluid albumin concentration in patients with ascites, shows a marked positive correlation (r = + 0 73, P < 0 001)   Albumin almost disappears from the fluid when the level in the plasma falls to 1 g per 100 ml and at higher levels the concentration in the plasma exceeds that in the fluid by about 1 g per 100 ml

   The relation between plasma and ascitic fluid albumin is further illustrated by the changes which occurred between the two observations on Case 18, which are shown in the following table

Date of observation	Plasma albumin %	Ascitic fluid albumin %
5 1 48	3 9	3 1
17 12 48	3 2	1 9

   Wolfson, Cohn, Calvary and Thomas (37) found, as mentioned above, that the albumin fraction obtained by Howe's method contains also the α-globulin, which varies in amount from 0 5–1 5 g per 100 ml , and tends to be higher with low values of plasma albumin   If α-globulin were confined to the plasma, the data of Fig 5 would be approximately accounted for by an equal partition of true albumin between plasma and fluid   This interpretation cannot be accepted unreservedly because Luetscher (20) has demonstrated α-globulin in pleural and ascitic fluid by electrophoresis   His values for plasma α-globulin contents, however, are substantially lower than those found by Wolfson, Cohn, Calvary and Thomas (37)

   The upper part of Fig 5 shows the relation between the albumin concentration of plasma and pleural fluid   A relation exists similar to that found in ascites (r = + 0 54, P < 0 05), the albumin concentration in the plasma exceeding that in pleural fluid by amounts varying from 0 5 to 2 4 g per 100 ml   The lower degree of correlation is due to the lack of patients with low plasma albumin levels

   The similarity of pleural and ascitic effusions in this respect is confirmed by the almost identical protein contents of fluids from different localities in patients with polyserositis

   Case 11 developed ascites, and six weeks later a right pleural effusion, which post-mortem examination revealed to be due to a reticulosis   The albumin level in the ascitic fluid was 2 7 g per 100 ml , and that in the

pleural fluid nine days later 2 5 g per 100 ml   The plasma albumin levels
on the two occasions were 3 2 and 3 4 g per 100 ml , respectively   In Case
16, ascites due to tuberculous peritonitis verified at operation was followed
after five months by a left pleural effusion   The albumin level in the ascitic
fluid soon after the ascites appeared was 3 1 g per 100 ml , and that in the
pleural fluid 3 4 g per 100 ml   During the interval of four months between
the observations the plasma albumin level had risen from 3 3 to 3 7 g per
100 ml
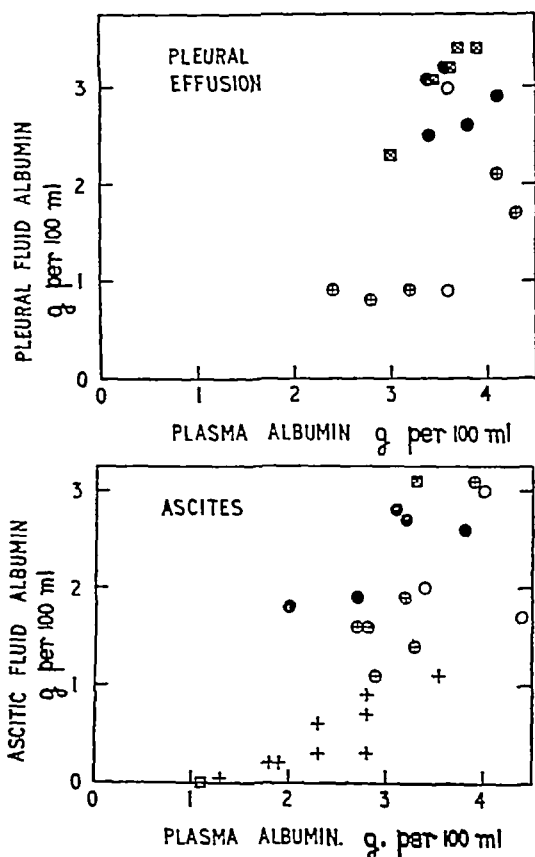
Fig 5   Shows the relation between plasma and fluid albumin level (g per 100 ml) in 17 patients
with pleural effusions and 24 with ascites   Symbols as in Figs 2 and 4

The tendency of different effusion fluids in the same patient to contain
equal amounts of protein was commented on by Luetscher (20) whose electro-
phoretic studies included some in four patients with multiple effusions   The
plasma protein level would seem to be the most likely equalising factor in
such patients

The correlation of plasma and effusion fluid albumin levels is most easily explained by supposing some at least of the capillaries in contact with the effusion to be permeable to albumin   In the case of ascites, there is some independent evidence that this is so   Mankin and Lowell (21) found that, when the plasma colloid osmotic pressure is increased by intravenous injection of albumin in a patient with ascites, the ascitic fluid osmotic pressure rose simultaneously (as judged by daily measurements) by an equal amount By whatever means they altered the osmotic pressure of plasma, such as by mercurial diuretics or by injection of saline, an equal change occurred in the osmotic pressure of the ascitic fluid

Several authors (2, 32) have drawn attention to the high protein content of thoracic duct lymph, and Peters (24) demonstrated the appearance of the dye T 1824, which is bound to serum albumin (29), in the thoracic duct of animals shortly after intravenous injection   Starling presented evidence that the protein in thoracic duct lymph came chiefly from the liver, and pointed out that the low capillary pressure in that organ makes permeability of the capillaries a necessity in order that tissue fluid should be formed at all   There is no direct evidence, however, that tissue fluid formed in the liver can mix with ascitic fluid, so that the site of leakage of plasma albumin into ascitic fluid cannot be said to have been located, although the occurrence of such leakage seems to follow from the observations recorded in Fig 5, and from those of Mankin and Lowell quoted above   This state of affairs has the consequence that the fluid albumin content is not a guide to the presence or absence of capillary damage, although, as will be seen below, inflammation of the serous linings does affect the albumin concentration of fluid to some extent

Pleural effusions are analogous to ascites in this respect, but no independent information seems to be available about the permeability of the pleural capillaries to albumin   The pleura is like the peritoneum in being supplied by more than one set of capillaries, those of the visceral pleura draining into the pulmonary veins (30) and it is likely that the pressure in these capillaries is lower than that in those of the systemic circuit, suggesting an analogy with the hepatic capillaries   The tendency for fluid to collect in these two serous cavities may possibly result from their being supplied by several sets of capillaries with differing permeability to plasma albumin

Fig 6 shows the relation between plasma and fluid globulin levels, which, except in tuberculous pleurisy, are not associated   The correlation coefficient between plasma and ascitic fluid globulin levels is — 0 22, which is not significant   That between plasma and pleural fluid globulin is + 0 64, which is significant (P < 0 01), but this is almost entirely due to the points representing patients with tuberculous pleurisy, so that it would be

misleading to compare the correlation for all pleural fluids with that for ascites, since the latter group only includes one patient with tuberculous peritonitis
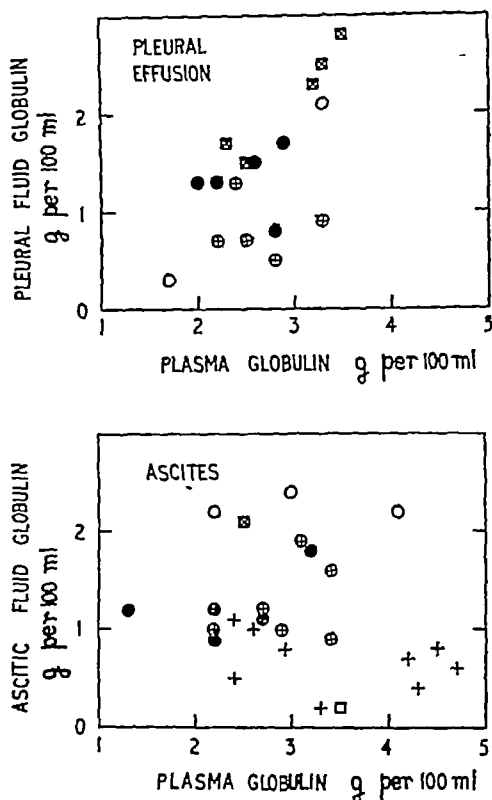
Fig 6   Shows the relation between plasma and fluid globulin level (g per 100 ml) in 17 patients with pleural effusions and 24 patients with ascites   Symbols as in previous Figs

Table V records the correlation coefficients between plasma and fluid protein, separately for pleural effusions and ascites, for heart failure, malignancy, tuberculosis and cirrhosis   This makes evident the association of plasma and fluid albumin, and the lack of such association of globulin level, except in tuberculous pleurisy when the association is well marked The significances of the correlation coefficients (but not their values) have been combined using the z transformation   This establishes that plasma and fluid albumin levels are correlated but globulin levels not, and this remains true if tuberculous pleurisy is excluded

These results are consistent with the view that simple diffusion is largely responsible for the albumin content of effusion fluids, both pleural and ascitic, but that, with the exception of tuberculous effusions, such a mechanism does not account for the concentration of globulin in effusion fluid

TABLE V

The correlation of plasma and fluid proteins for each diagnosis

			Correlation coefficient	Number of patients	P	$z^2(N-3)$
Albumin	Ascitic	Heart failure	+0 81	6	< 0 05	3 83
		Carcinoma	+0 80	5	> 0 1	2 38
		Cirrhosis	+0 87	9	< 0 01	10 61
	Pleural	Heart failure	+0 89	5	< 0 05	3 92
		Carcinoma	+0 19	5	> 0 1	0 07
		Tuberculosis	+0 95	5	< 0 02	6 41
					$\chi^2 =$	27 22
					P	< 0 001
Globulin	Ascitic	Heart failure	+0 34	6	> 0 1	0 390
		Carcinoma	+0 55	5	> 0 1	0 768
		Cirrhosis	—0 32	9	> 0 1	0 696
	Pleural	Heart failure	—0 08	5	> 0 1	0 014
		Carcinoma	+0 06	5	> 0 1	0 008
		Tuberculosis	+0 96	5	< 0 01	7 606
					$\chi^2 =$	9 482
					P =	0 15

Qualitatively similar results were obtained by Luetscher (20), who employed electrophoretic methods, and recorded the total protein content of plasma and effusion fluid, and percentage composition with respect to the different fractions   If the absolute values of each protein are calculated from his figures, and α-globulin included in the " albumin " fraction, relations between plasma and fluid protein similar to those found here are obtained, within the limits imposed by the small number of cases investigated

(2) *The nature of the effusion*

The general supposition that inflammatory diseases cause effusion of fluids containing large amounts of protein, in contrast to congestive failure, cirrhosis and nephrosis, finds confirmation in the present work   Table VI gives the mean levels of effusion fluid proteins for different diseases

Fluids caused by malignancy and tuberculosis contained more protein than those caused by heart failure   The difference is less conspicuous in ascitic fluid than in pleural, where there was no overlapping of the individual values for total protein found in heart failure and in tuberculosis   Andrews,

Pickering and Sellors (1) found total fluid protein of no value in detecting the presence of tuberculous pleurisy or peritonitis in patients with tuberculous polyserositis and constrictive pericarditis, but they point to the possibility of an effusion in such a patient being predominantly caused by venous congestion, even if tuberculous inflammation is present. Of the two patients in the present series with pleural effusions due to tuberculous polyserositis with constrictive pericarditis, one, Case 7, died 3 months after the observation was made, and post-mortem examination revealed tuberculous inflammation of all serous cavities. In the other, Case 6, although tubercles were seen in the peritoneum by peritoneoscopy, thoracoscopy revealed neither tubercles nor thickening of the pleura. The total fluid proteins, 5 1 g per 100 ml in Case 7 and 1 2 g per 100 ml in Case 6 favour the interpretation of the former effusion as tuberculous and the latter as congestive.

TABLE VI

The mean fluid protein level (g per 100 ml) in different diseases

	Pleural		Ascitic	
	Albumin	Globulin	Albumin	Globulin
Heart failure	1 3	0 8	1 8	1 3
Constrictive pericarditis	1 9	1 2	2 2	2 3
Carcinoma	2 9	1 3	2 4	1 2
Tuberculosis	3 1	2 2	3 1	2 1
Nephrosis	—	—	0	0 2
Cirrhosis	—	—	0 5	0 7

The intimate association of globulin levels in plasma and fluid of tuberculous origin supports strongly the traditional contention that such inflammation renders the capillaries abnormally permeable to protein.

The association of hypoproteinæmia and effusion

The levels of plasma protein in the patients studied in this investigation are shown in Figs 1 and 3, to be usually below normal. Only two out of 25 patients with ascites, and three out of 17 with pleural effusions had a plasma albumin greater than 4 g per 100 ml, in contrast to six healthy subjects whose plasma albumin varied from 4 0 to 4 7 g per 100 ml with a mean of 4 2.

Table VII compares the mean plasma colloid osmotic pressures of six normal subjects with that of patients with effusions. Although the lowest plasma osmotic pressures are found in patients with cirrhosis, low values

are common enough in patients with effusions due to other causes, and the mean plasma osmotic pressures of patients with cirrhosis is not significantly lower than that of patients with ascites of other origin (P = 0 07)

TABLE VII

Mean plasma osmotic pressure (in cm of water) of healthy subjects and patients with effusions

	Mean plasma osmotic pressure	No of subjects
Normal	32 9	6
Pleural effusion (all causes)	26 2	17
Ascites (except cirrhosis and nephrosis)	24 5	15
Ascites due to cirrhosis	21 0	9

Hypoproteinæmia has commonly been supposed to be a major cause of the ascites of cirrhosis (22, 33, 27, 13) and a subsidiary cause of the effusions and œdema of congestive heart failure (26, 12)   Such suppositions must be viewed with suspicion if plasma and fluid albumin concentration are related as demonstrated earlier in this paper , for moderate reduction in the plasma albumin level would then be reflected in a lower effusion fluid albumin level, and the osmotic pressure difference, which is the osmotic quantity on which fluid exchange depends, would remain unaltered   Such considerations do not, of course, apply to severe hypoproteinæmia such as that encountered in nephrosis, where there is reason for suspecting a qualitative change in the " albumin " fraction   in such a patient, as in Case 30, hypoproteinæmia can of itself cause effusion in serous cavities

Hypoproteinæmia might at least as reasonably be regarded as the result of effusions as their cause   The mean total plasma protein was lower (5 3 g per 100 ml) in patients with ascites due to neoplasm than in those with pleural effusion due to the same cause (6 0 g per 100 ml), which might be a reflection of the greater volume of fluid which the peritoneal cavity is capable of containing

Myers and Keefer (22) saw no progressive reduction of plasma proteins during a period of repeated paracentesis, and the same phenomenon, together with the ability of dogs to regenerate protein removed by plasmapheresis led Barnett, Jones and Cohn (3) to reject loss of protein into ascitic fluid as a cause of hypoproteinæmia

Payne and Peters (26) ascribed the hypoproteinæmia of congestive failure to malnutrition caused by anorexia, and the same cause may well be at work in many of the patients in the present series

The mean plasma osmotic pressure of two patients with tuberculosis, one patient with congestive failure, and one patient with bronchial carcinoma, none of whom had effusions, was 28 3 cm , one value being as low as 25 4 cm   Reduction in the plasma osmotic pressure is probably a common occurrence in serious disease, but it would require a large survey to discover whether such reduction is particularly great in patients with collections of fluid

SUMMARY

1   The antecubital venous pressure and the effusion fluid pressure have been measured simultaneously in patients with pleural effusions or ascites due to various causes

2   The albumin and globulin content of plasma and of effusion fluid have been determined in each patient by the micro-Kjeldahl method, and the colloid osmotic pressures calculated

3   In patients with pleural effusion, the venous pressure always exceeded the pleural pressure   The difference between the two was approximately equal to the difference between the colloid osmotic pressures of plasma and fluid

4   In 15 out of 16 patients with ascites due to causes other than cirrhosis, the venous pressure exceeded the ascitic pressure   the difference between the two was proportional to the difference between the colloid osmotic pressures of plasma and fluid

5   In patients with ascites due to cirrhosis this proportionality was not observed   in 7 out of 9 patients the ascitic pressure exceeded the venous pressure, while the difference between the colloid osmotic pressures of plasma and ascitic fluid was large owing to the low protein content of the latter

6   It is suggested that portal obstruction accounts for the different behaviour of ascites due to cirrhosis in this respect, and that the magnitude of the deviation provides a measure of the portal pressure

7   Portal pressures estimated in this way are comparable with those measured directly by other workers on patients with cirrhosis , the highest estimates were found in patients who had had hæmatemeses

8   The albumin content of effusion fluid was proportional to that of plasma   the globulin contents were not related except in tuberculous pleurisy   Effusion fluids caused by tuberculosis and carcinoma contained more protein than those caused by congestive failure

9   The fluid protein contents were approximately consistent with the view that albumin enters effusion fluid largely by simple diffusion, but that globulin only does so where capillary permeability is increased by inflammation

10   Hypoproteinæmia is usually found in patients with effusions, being more severe in those with ascites, particularly if this is caused by cirrhosis of the liver   The interpretation of this finding is discussed

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CLINICAL SCIENCE

AUTHORS

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THE ACTION OF OUABAIN (G-STROPHANTHIN) ON THE CIRCULATION IN MAN, AND A COMPARISON WITH DIGOXIN

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THE mode of action of the cardiac glycosides in cardiac failure is still debatable and the clinical results unpredictable   Certain patients show remarkable clinical improvement, while others fail to respond   In the previous generation it was thought that the best responses were obtained in auricular fibrillation because the ventricular rate was markedly slowed, but the frequent occurrence of clinical improvement in patients with sinus rhythm suggested, in addition, a stimulating action on the myocardium of the failing heart   Even the latter hypothesis is not universally applicable as clinical improvement does not always follow digitalisation   Previous work in this school (8, 9) showed that venous pressure reduction was sometimes independent of any upward change in cardiac output and it was tentatively suggested that the primary action of digoxin might be to reduce the venous pressure   Comparison of the effects of digoxin and venesection in various types of heart failure (8) showed a certain parallelism   In " low output " failure, venesection and digoxin both raised the cardiac output while, in the " high output " type (e g , emphysema heart), the minute volume of the circulation might fall as a result of these measures   Further work, however, showed that primary venous pressure reduction could not adequately account for the action of digoxin   It was found that cardiac work was greater after digoxin than after venesection (8), and that certain rather exceptional types of raised venous pressure were not lowered by digoxin (14)   Ouabain, moreover, was found by Bloomfield and his colleagues (2) to increase the cardiac output in many instances of heart failure without reducing the venous pressure at all   It was therefore decided to repeat studies on cardiac output responses in healthy and failing hearts using ouabain, and to make further comparative observations with digoxin

Material and Methods

Ouabain was given to nine subjects with normal circulation and to twenty-eight with heart disease of different types   Thirty-two patients with cardiac failure of varied aetiology were treated with digoxin   The

observations were begun two hours after the midday meal   Normal subjects were recumbent, but most of the cardiac patients were propped up at an angle of 45 degrees   A catheter was passed into the right auricle, and the techniques employed were the same as in previous studies, except that the Haldane blood-gas apparatus used to determine the oxygen unsaturation of right auricular and arterial blood was completely immersed in a water-bath to ensure full temperature control (5)   Three ml samples of blood were used in these analyses   Arterial blood was assumed to be 95 per cent saturated with oxygen in normal subjects , in patients with heart failure arterial samples were taken for direct determination of oxygen saturation The mean right auricular pressure was measured with a saline manometer, taking the sternal angle as zero   Oxygen consumption was measured by spirometry   In six subjects the effects of lowering the venous pressure by congesting cuffs round the thighs were compared with the results obtained with ouabain

In plotting the effects of ouabain and digoxin on patients with hypertensive and ischæmic heart disease (Fig 4), a logarithmic scale is used to indicate proportional changes in cardiac output , thus a rise in output from 2 to 3 litres per minute represents the same degree of change as a rise from 4 to 6 litres   Random errors in the measurement of smaller arterio-venous oxygen differences in the higher ranges of cardiac output are placed in proper perspective by this technique   Venous pressure changes are not liable to the same error and are recorded on a linear scale

To ensure a maximum response, 1 mg of ouabain was used in the earlier observations, and on one occasion 2 mg were given in error   These large doses caused unpleasant effects, subjectively in the form of headache, and objectively in hypertension   In later studies, the usual dose was 0 75 mg and even this might cause transient hypertensive reactions   The subjective sensations were less, however, if the drug was injected very slowly over a period of five minutes   In undersized subjects doses of 0 5 mg were used

RESULTS

The effects of ouabain are shown in Tables I-III, in which the right auricular pressure, cardiac output, blood pressure and heart rate are recorded before and after administration of the glycoside   The early changes, which represent the first significant alteration in either right auricular pressure or cardiac output, are also shown, and the time in minutes after ouabain when these changes occur is recorded in italics   The final change represents the last observation made before the experiment was terminated, usually 45-60 minutes after the glycoside was given,   Details of selected cases are given in Figs 1-3

TABLE I

GROUP I *Effects of ouabain on patients with normal circulation*

Case No	Diagnosis	R.A.P cm. saline			O.O litres per min.			% Final change in O.O	% Final rise in O.O per 1 cm fall in R.A.P	Blood pressure			Heart rate			Remarks
		Initial	Early	Final	Initial	Early	Final			Initial	Early	Final	Initial	Early	Final	
1	Pernicious Anaemia, Subacute combined degeneration	+1.0	+0.5 (3)	+0.5	5.3	5.0	5.3	0	0	120/80	120/80	120/80	80	80	80	·
2	Carcinoma of stomach	−4.5	−3.0 (8)	−4.5	7.2	6.8	5.8	−10.4	—	140/70	145/72	140/76	72	62	70	
3	Subarachnoid haemorrhage	−6.0	−7.5 (8)	−8.5	6.1	8.3	6.8	+11.5	+4.4	130/80	135/82	108/78	70	108	82	2 mg ouabain
4	Convalescent pneumonia	+1.5	−0.5 (5)	−1.0	5.8	5.1	6.5	+12.1	+4.8	120/75	140/85	138/80	84	72	80	
5	Convalescent tonsillitis	+1.0	+1.5 (3)	+0.5	9.7	9.1	8.3	−14.4	−28.8	130/70	135/70	135/75	72	68	68	
6	Paraplegia	+2.5	+4.0 (5)	+5.0	4.3	4.5	4.6	+4.7	+9.4	170/90	165/96	108/98	78	68	70	
7	G.P.I.	−7.0	−7.5 (7)	−8.0	5.1	4.8	5.2	+1.9	+1.9	110/70	130/80	125/75	80	68	70	
8	Steatorrhœa	−4.0	−3.0 (7)	−0.0	8.0	11.2	8.4	−2.3	−1.6	95/68	135/90	105/70	88	92	84	
9	Syringomyelia and chronic bronchitis	−11.0	−11.5 (9)	−11.0	4.5	6.0	6.3	+17.7	0	149/90	140/85	149/90	88	80	74	

I —Effect of ouabain on cardiac output and right auricular pressure

Patients with normal circulation (Group I, Table I)   In patients with a normal circulation ouabain produced no consistent change in cardiac output   There was perhaps a slight tendency for the right auricular pressure to fall but this was not outside the limits of normal fluctuations
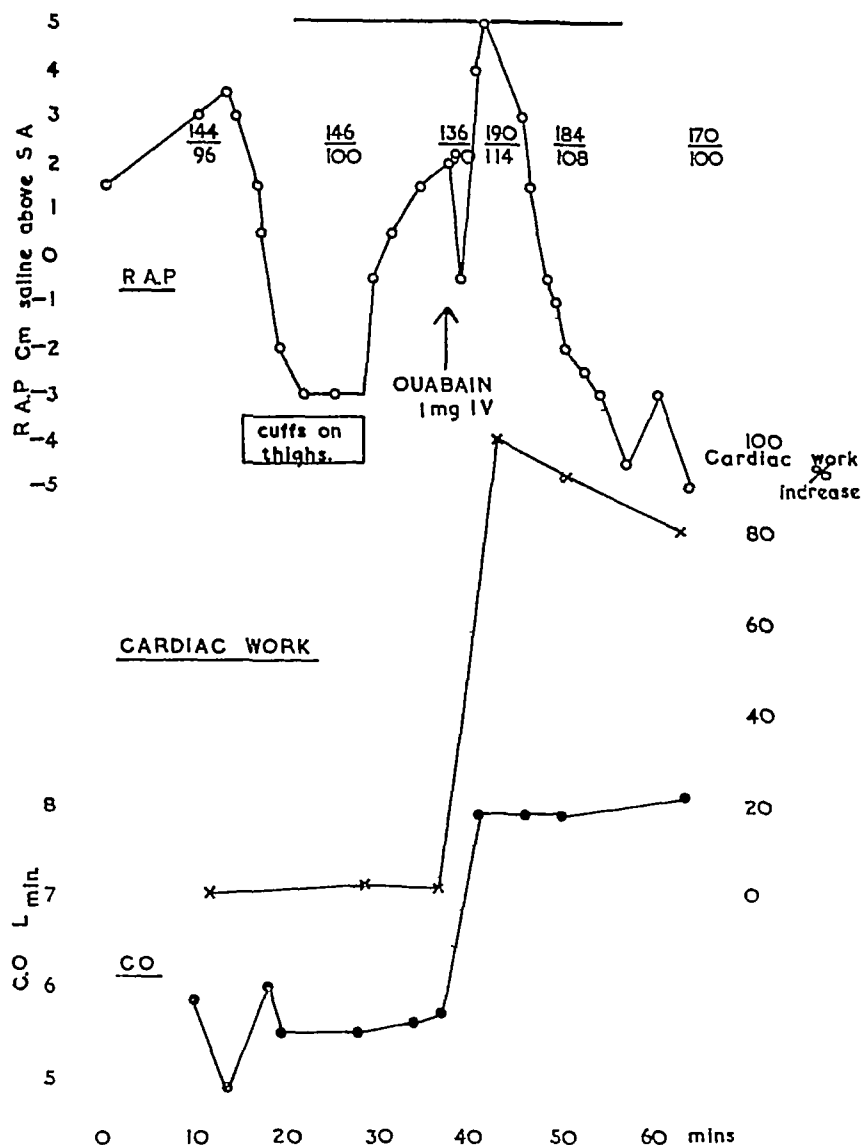
Fig 1   Case 29   Hypertensive heart disease   Mechanical reduction of the right auricular pressure produced an insignificant change in cardiac output   Ouabain produced a rise in cardiac output coincident with a rise in venous pressure and a hypertensive effect   Later when the venous pressure had fallen, the cardiac output was much higher than after mechanical reduction of the venous pressure

Patients with heart disease without failure (Group II, Table II) Two patients had heart disease but were not, at the time of the observations, in failure Ouabain produced no significant change in cardiac output or venous pressure, i e , these patients responded in a similar manner to those with a normal circulation
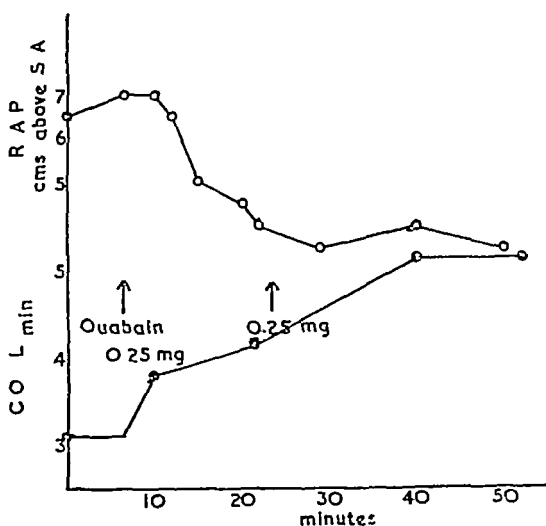
Fig 2   Case 22   Hypertensive heart disease   Ouabain produced a rise in cardiac output before any significant fall in venous pressure

Patients with low output failure (Group III, Table II) Four of six patients with valvular disease of the heart showed no significant rise in cardiac output with ouabain , all those with hypertensive or ischæmic heart disease, on the other hand, showed an immediate rise accompanied, in half the cases, by a venous pressure fall In four instances (Cases 22, 23, 27, 29) there was a significant rise in cardiac output before the venous filling pressure had fallen at all , sometimes the venous pressure rose simultaneously with the rise in output (Fig 1) In contrast, digoxin never produced a rise in output without a simultaneous fall in right auricular pressure (Table V) Patients with pulmonary congestion without signs of systemic venous engorgement (Group IV, Table II) showed a similar response to those with low output failure and a raised venous pressure The right auricular pressure, initially within normal limits, fell following ouabain and there was an increase in cardiac output

The effect of ouabain on the cardiac output was compared with that produced by congesting cuffs round the thighs (Table IV) Ouabain produced a greater rise in output for each cm fall in venous pressure than did the cuffs, except in one patient with mitral stenosis (Case 16)

TABLE II

GROUP II *Effect of ouaban on patients with heart disease not in failure*

Case No	Diagnosis	R.A.P on saline			C O litres per min.			% Final change in C O	% Final rise in C O per 1 cm fall in R.A.P	Blood pressure			Heart rate			Remarks
		Initial	Early	Final	Initial	Early	Final			Initial	Early	Final	Initial	Early	Final	
10	Aortic aneurysm Auricular fibrillation	0 0	−0 5 (4)	−1 5	5·1	4 8	4 5	−11 7	−7 8	112/58	120/60	124/65	130	130	110	
11	Mitral stenosis Pulmonary haemosiderosis	−2 0	−2·0 (3)	−1·0	0 0	5·2	0·0	0	0	—	—	—	50	48	44	

GROUP III *Effect of ouaban on patients with low output failure*

(a) Valvular disease

Case No	Diagnosis	R.A.P on saline			C O litres per min.			% Final change in C O	% Final rise in C O per 1 cm fall in R.A.P	Blood pressure			Heart rate			Remarks
		Initial	Early	Final	Initial	Early	Final			Initial	Early	Final	Initial	Early	Final	
12	Aortic stenosis	+12 5	+10 0 (6)	+5·5	2·4	2 8	3 1	+29 1	+4 1	100/90	120/105	115/95	120	128	120	
13	Mitral stenosis Auricular fibrillation	+0 5	+7 5 (6)	+6 0	2 8	3 0	4 5	+60·4	+17 1	120/104	130/106	140/103	50	52	60	
14	Mitral stenosis	+5 0	+1 5 (2)	+3 5	4·6	4·4	4 3	−0 5	−4 3	135/85	140/90	150/90	72	98	64	
15	Aortic incompetence	—	— (10)	—	2 0	2 9	2 0	0	—	100/76	180/74	180/70	96	96	96	Right ventricular pressures only
16	Mitral stenosis Auricular fibrillation	+5·0	+3 5 (5)	−1 5	1 7	1 8	1 0	+11 8	+1 8	100/70	110/78	110/70	150	140	121	Cuffs.
17	Aortic stenosis	−9·0	−10 0 (9)	−10 5	4·0	4·1	4 4	+10·0	+1 0	100/60	115/60	108/90	96	98	96	

(b) *Hypertensive and ischaemic heart disease*

18	Myocardial Infarction	+1·5	−1·5 (10)	−5·5	2·75	3·3	1·0	+15·5	+0·5	120/80	1·0/105	130/80	1·0	120	1·0	
19	Hypertensive heart disease	+2·5	−3·5 (13)	−1·5	2·6	3·8	4·4	+69·0	+0·9	105/135	216/135	215/140	110	106	101	
20	Ischaemic heart disease	+9·0	+6·0 (17)	+6·0	3·35	4·0	4·0	+37·3	+0·3	110/90	160/88	160/88	88	84	81	
21	Hypertensive heart disease Auricular fibrillation	+16·5	+16·0 (4)	+12·5	2·75	3·1	3·3	+20·0	+0·7	170/125	200/120	190/125	86	92	90	
22	Myocardial Infarction	+0·5	+7·0 (3)	+4·0	3·1	3·8	5·2	+67·8	+27·2	170-1·60 / 130	275-170 / 130	200 195 / 135	10·0	105	10·0	Fig 2
23	Hypertensive heart disease Auricular fibrillation	+17·5	+17·0 (16)	+16·0	4·0	5·3	5·4	+17·4	+7·0	170/1·0	164/120	160/1·0	140	110	128	
24	Ischaemic heart disease	+1·5	−1·0 (18)	−3·0	1·0	1·8	2·5	+50·2	+12·5	115/80	154/08	172/08	111	136	115	Weight 70 lbs.
25	Hypertensive heart disease	−2·5	−1·5 (18)	−7·0	3·0	3·3	3·6	+20·0	+1·1	110/130	200/170	210/138	80	90	90	Cuffs.
26	Hypertensive heart disease	−1·5	−0·5 (15)	−2·5	2·2	2·2	2·5	+13·0	+13·0	160/100	150/102	160/100	92	90	89	Cuffs.
27	Hypertensive heart disease Aortic stenosis Auricular fibrillation	+0·0	+5·0 (15)	+1·0	2·0	3·1	3·3	+27·0	+27·0	200/100	192/85	220/95	81	70	68	Cuffs.
28	Hypertensive heart disease	+10·0	+4·0 (11)	+2·0	2·4	2·8	3·4	+11·7	+5·2	145/105	160/110	160/110	118	112	112	
29	Hypertensive heart disease Bronchitis	+1·6	+6·0 (4)	−5·0	5·05	7·0	8·2	+16·2	+6·06	130/90	100/114	170/100	208	104	103	Arterial blood 87% saturated. Cuffs. Fig 1

GROUP IV   *Effect of ouabain on patients with pulmonary congestion without systemic venous engorgement*

30	Hypertensive heart disease	−0·5	−12·0 (9)	−12·0	2·75	1·1	4·1	+40·0	+10·6	185/110	101/125	101/110	68	70	72	
31	Hypertensive heart disease Bronchitis	−8·0	−7·0 (7)	−8·5	3·9	4·8	5·0	+28·2	+57·4	150/90	190/100	100/95	98	84	84	Arterial blood 89% saturated.
32	Hypertensive heart	−8·0	−9·0 (7)	−10·0	7·0	7·8	7·8	+11·1	+5·7	320/110	260/135	320/105	71	84	80	Cuffs

TABLE III

GROUP V   *Effect of ouabain on patients with emphysema heart (high output failure)*

Case No	Diagnosis	R.A.P cm saline			C.O litres per min.			% Final change in C.O	% Final rise in C.O per 1 cm fall in R.A.P	Blood pressure			Heart rate			Remarks
		Initial	Early	Final	Initial	Early	Final			Initial	Early	Final	Initial	Early	Final	
33	Emphysema	−3.5	−1.0 (6)	−6.0	6.5	7.2	5.1	−21.5	−8.6	140/90	185/105	100/100	86	84	72	Arterial blood 83% saturated
34	Chronic bronchitis and emphysema	−2.5	—	−3.5	5.05	—	6.8	+34.7	+34.7	130/80	—	160/90	76	—	76	Fig 3 Arterial blood 80% saturated
35	Chronic bronchitis	+7.0	+3.0 (7)	0.0	7.0	8.7	9.2	+31.4	+4.5	95/55	90/55	95/50	96	90	90	Arterial blood 81% saturated.
36	Chronic bronchitis	0.0	0.0 (11)	−1.5	4.56	5.3	6.3	+38.4	+25.6	152/96	100/78	160/92	84	96	96	Arterial blood 84% saturated
37	Kypho-scoliosis Emphysema	+3.5	+5.0 (15)	0.0	7.7	8.8	11.4	+48.0	+13.7	168/105	176/110	160/100	78	92	78	Arterial blood 59% saturated.

Patients with emphysema heart (high output failure)   *(Group V, Table III)*   Of five patients with emphysema heart, four had an increase in cardiac output after ouabain, which was accompanied by a relatively small fall in venous pressure   It should be noted that the apparent degree of
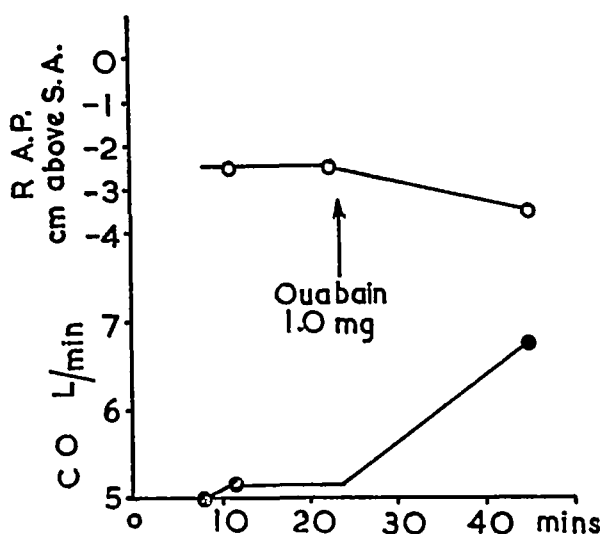
Fig 3   Case 34   Emphysema heart disease   Ouabain produced a rise in cardiac output without a significant fall in the venous pressure

venous congestion was less in the patients of this group than in those with hypertension, but it must be remembered that the right auricular pressure in uncomplicated emphysema is often low in relation to the sternal angle,

TABLE IV

Effect of congesting cuffs on the thighs

Case No	CUFFS			OUABAIN		
	Fall in R.A.P (cms)	% change in C O	% rise in C O per 1 cm fall in R A.P	Fall in R.A.P (cms)	% change in C O	% rise in C O per 1 cm fall in R.A P
16	4	+8 8	+2 2	6 5	+11 8	+1 8
25	5	+11 3	+2 2	4 5	+20 0	+4 5
26	4	+17 6	+4 4	1 0	+13 6	+13 6
27	3 5	+24 0	+6 9	1 0	+27 0	+27 0
29	6 0	+1 85	+0 30	6 5	+45 0	+6 9
32	2 5	—11 4	—4 55	2·0	+11 4	+5 7

TABLE V

The effect of digoxin on patients with low output failure

(a) *Valvular disease*

Case No	Diagnosis	R.A.P cm saline			C.O litres per min			% final change in CO	% final rise in CO per 1 cm fall in R.A.P
		Initial	Early	Final	Initial	Early	Final		
38	Mitral stenosis Aortic incompetence Auricular fibrillation	0 0	−3 0 (10)	−4 5	3 85	4 2	5 8	+50 5	+11 2
39	Mitral stenosis Auricular fibrillation	+6 0	−1 5 (20)	−3 0	2 5	2 85	3 3	+32 0	+3 6
40	Aortic incompetence	−2 5	−3 5 (10)	−5 5	3 15	3 3	3 0	+24 0	+8 0
41	Mitral stenosis Aortic incompetence	+7 5	+2 5 (8)	−2 5	2 3	2 1	3 8	+65 0	+6 5
42	Mitral stenosis	+16 0	+12 0 (25)	+11 0	3 1	3 4	3 6	+16 0	+3 2
43	Mitral stenosis Aortic stenosis	+6 5	+1 5 (10)	−1 5	2 95	3 25	4 15	+40 5	+6 0
44	Mitral stenosis	+21 0	+18 0 (7)	+11 5	2 45	2 55	3 15	+28 6	+3 0
45	Mitral stenosis Auricular fibrillation	+8 5	+4 5 (16)	+4 5	3 2	3 65	4 5	+40 5	+10 1
46	Mitral stenosis Aortic incompetence	+5 0	+2 0 (27)	+1 5	2 5	2 5	2 6	+4 0	+1 1
47	Mitral stenosis	+1	−2 0 (17)	−3 0	4 15	5 5	6 9	+66 3	+16 6
48	Aortic stenosis	+13 5	+10 0 (18)	+9 0	3 45	3 55	4 0	+16 0	+3 6
49	Aortic incompetence	−5 0	−7 5 (11)	−7 5	3 95	4 1	4 3	+8 9	+5 0
50	Mitral stenosis	+21 0	+17 5 (7)	+14 0	3 1	3 15	3 5	+12 8	+1 8
51	Aortic incompetence Auricular fibrillation	+6 0	+6 0 (6)	+5 5	2 85	2 95	3 2	+12 0	+2 4

(b) Hypertensive and ischæmic heart disease

52	Hypertensive heart disease	+10 0	+16 0 (25)	+15 0	3 8	4 7	5 0	+31 0	+7 9
53	Hypertensive heart disease	+16 0	+12 0 (19)	+12 0	3 0	3 2	3 3	+10 0	+2 5
54	Myocardial infarction	+24 0	—	+20 0	4 7	—	5 1	+8 5	+2 1
55	Hypertensive heart disease Auricular fibrillation	+5 0	+2 5 (8)	+2 0	4 6	4 6	5 0	+8 7	+3 0
56	Hypertensive heart disease	+18 0	+14 0 (12)	+6 0	2 3	2 6	2 9	+26 1	+2 2
57	Hypertensive heart disease	+7 0	—	+3 0	2 85	—	3 0	+5 3	+1 3
58	Hypertensive heart disease	+8 0	+3 0 (13)	0 0	3 05	3 2	5 3	+74 0	+9 2
59	Hypertensive heart disease	+4 0	+1 5 (12)	—1 0	3 2	3 35	5 1	+50 5	+10 0
60	Hypertensive heart disease	+0 5	+1 0 (19)	—2 0	3 1	3 6	4 5	+45 0	+3 0
61	Hypertensive heart disease	+13 0	+0 0 (13)	+0 0	3 0	2 9	2 0	—3 2	—0 8
62	Hypertensive heart disease	+16 0	—	+9 0	2 2	—	2 2	0 0	0 0
63	Malignant hypertension	—4 0	—7 0 (12)	—12 0	2 1	2 5	2 7	+28 6	+3 6

owing to elevation of the latter and the low position of the diaphragm (3, 10)
Venous pressures in the neighbourhood of the sternal angle in such patients
certainly indicate significant congestion   In all but one of this group the
effect of ouabain was to increase the cardiac output conspicuously, with a
small accompanying fall in venous pressure
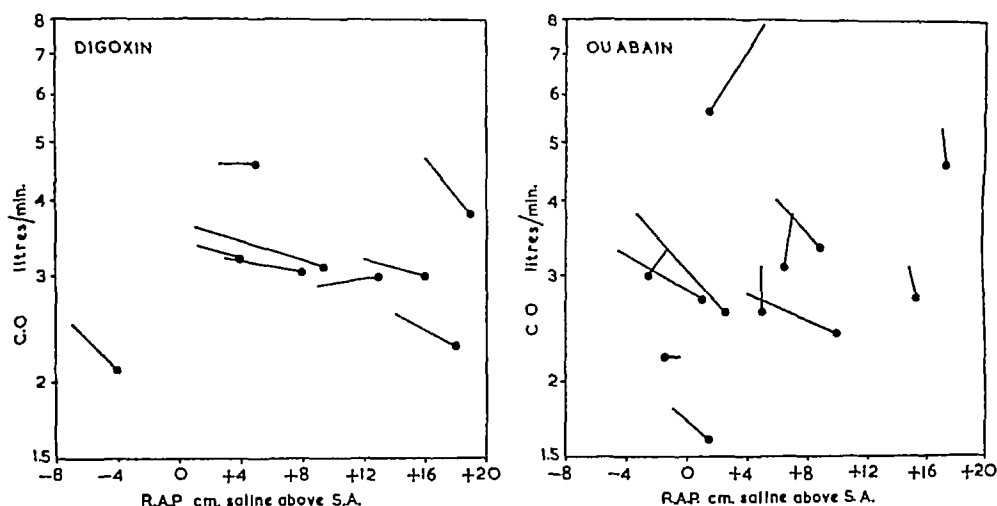
Fig 4   Hypertensive heart disease, showing the early changes in cardiac output and right
auricular pressure after digoxin and ouabain   With digoxin there is a significant fall in
venous pressure before any significant rise in cardiac output   With ouabain a steep rise
in cardiac output with little change in venous pressure is common

II —*Other observations on ouabain*

The speed of action   Ouabain given through an intracardiac catheter
usually produces an effect within ten to fifteen minutes, and attains its
maximum effect within half-an-hour   Digoxin given in a similar way may
not begin to act for twenty minutes and exerts a maximum effect within
thirty to forty-five minutes

The effect on pulsus alternans   One patient (Case 22) with hypertensive
heart disease had striking pulsus alternans   Before ouabain the systolic
pressure of alternate beats differed by 20 mm Hg   (170-150/130) and almost
immediately after ouabain the alternation was barely measurable (200-
195/135)

Hypertensive effects   In nearly half the cases, ouabain was followed
within five minutes by a rise in arterial pressure of more than 20 mm Hg
systolic or 10 mm Hg diastolic   This was sometimes associated with
transient but unpleasant symptoms, comprising headaches, visual dis-
turbances and paraesthesiæ, and even temporary exacerbation of breathless-
ness   In some of these cases there was a rise in venous pressure during the
hypertensive response, but despite this the cardiac output increased (Fig 1)

TABLE VI

Effect of digoxin on patients with emphysema heart (high output failure)

Case No	Diagnosis	R.A P cm saline			CO litres per min			% final change in CO	% final rise in CO per 1 cm fall in RAP
		Initial	Early	Final	Initial	Early	Final		
04	Emphysema bronchitis	+7.5	+3.0 (5)	+1.5	0.4	4.4	4.2	−31.4	−5.7
05	Emphysema bronchitis	+5.0	—	−1.0	7.7	—	6.0	−22.0	−3.7
06	Emphysema bronchitis	−0.5	—	−10.5	0.0	—	5.7	−5.0	−1.2
07	Emphysema bronchitis	+12.0	—	+5.0	0.1	—	5.4	−11.4	−1.0
08	Emphysema bronchitis	+12.0	+5.5 (11)	+4.5	9.0	8.4	8.0	−11.1	−1.5
00	Emphysema bronchitis	0.0	+0.5 (11)	−1.5	7.4	6.3	5.7	−23.0	−15.0

TABLE VII

Comparison of the effects of digoxin and ouabain on patients with cardiac failure

CONDITION	DIGOXIN			OUABAIN			
	No of cases	Mean rise in CO (percentage) per 1 cm fall in RAP	Standard error of mean	No of cases	Mean rise in CO (percentage) per 1 cm fall in RAP	Standard error of mean	"t" test of significance
Low output failure							
Valvular disease	14	+5.9	1.18	0	+3.8	2.05	Not significant (0.58)
Hypertensive and ischaemic heart disease	12	+3.8	1.03	15	+14.0	3.64	Significant (2.85)
High output failure	0	−4.8	5.3	0	+13.8	7.4	Significant (2.3)

III —*Comparison of the action of ouabain and digoxin*

Tables V and VI summarise previously published observations on the action of digoxin and additional observations on new cases   Special emphasis has been laid on the earliest measurable response   With digoxin it is seen that a significant reduction of right auricular pressure is nearly always the first reaction and the cardiac output measured simultaneously shows usually only a small increase   The early responses to ouabain and digoxin in cases of hypertensive and ischæmic heart disease are plotted in two graphs side by side (Fig 4)   In this group it is seen that the early response to ouabain is usually a striking rise in cardiac output with only a slight fall in venous pressure while with digoxin the earliest response is a fall in venous filling pressure with only a very slight rise in cardiac output

In Table VII the complete effects of digoxin and ouabain on the cardiac output are shown in relation to the accompanying venous pressure change   Measured in this manner it is seen that in the hypertensive and ischæmic heart disease group, taken because of conveniently comparable conditions and numbers, there is a greater relative rise in cardiac output after ouabain than after digoxin

DISCUSSION

The above data and the work of others (2) have shown that ouabain has a striking stimulating action on certain types of failing myocardium, and a rise in cardiac output may occur in such patients independently of a fall in venous pressure although the latter usually follows   Ouabain, however, has no significant or measurable effect on the output of a normal heart   Further observations with digoxin continue to show in nearly all cases, a venous pressure lowering action which has been confirmed by other workers (11)   This fall in venous pressure may precede significant increase in cardiac output, and in some cases may occur without any measurable rise in output during the period of observation

It has long been claimed on the European continent that strophanthin and ouabain often produce clinical improvement when digitalisation had apparently failed (6, 12, 13)   In the English speaking countries, however, the action of the cardiac glycosides is assumed to be identical   This seems to date from an observation by Cushny (4) who in 1897 said   " The action of all of them (various glycosides) on the cardiac muscle is identical in kind, although varying in strength   Antiarin and strophanthin were the strongest   "   We have not found any experiments in the literature in which a definite comparison of digitalis and strophanthin was made   No doubt this results from the difficulty in producing a standard degree of failure in an animal heart and the impossibility of repeating an experiment of this type under identical conditions   There has, however, been a strong pre-dilection on the part of the laboratory pharmacologists to use strophanthin experiments to illustrate the stimulating action of cardiac glycosides on the heart, perhaps because of their more rapid action (1, 7)

The studies reported here make it clear that the direct action of ouabain, increasing the output of the failing heart, is much easier to demonstrate than any similar action of digoxin   The frequency with which a rise in cardiac output was seen in the high output failure group contrasts with the frequent absence of any such response following digoxin in similar cases   Both drugs, however, may ultimately produce similar end-results, and it would be premature at the present time to claim fundamental differences of action   There is certainly a difference in the timing of the effects, ouabain producing a more rapid response   The stimulating action of ouabain probably brings about a fall in venous pressure as a secondary effect   Digoxin produces an early fall in venous pressure, which at this stage may be independent of any significant increase in cardiac output

SUMMARY

The action of ouabain has been studied in nine subjects with normal circulation and in twenty-six patients with various types of heart failure   The results are compared with thirty-three similar studies made with digoxin

When the circulation is normal, ouabain produces no significant effect on cardiac output or venous filling pressure   In cases with heart failure there is an increase in cardiac output which is often the first response, and which may be followed later by a fall in venous pressure

The total effect of digoxin is similar, but quantitatively and in timing, there are differences   The venous pressure tends to fall more in proportion to the change in cardiac output, and the fall in venous pressure often precedes a significant change in cardiac output   With ouabain the cardiac output goes up before there has been much change of venous pressure

Ouabain often produces an increase in cardiac output in emphysema heart failure—a reaction commonly absent after digoxin

In individual cases the action of ouabain on the cardiac output in heart failure is greater than that produced by mechanical reduction of venous pressure, and must therefore depend on a direct stimulating action on the failing myocardium

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INTRAPULMONARY MIXING OF HELIUM
IN HEALTH AND IN EMPHYSEMA

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IF an inert gas is rebreathed from a spirometer, gaseous equilibrium between lungs and spirometer will, after a while, be reached, and from the fall in the percentage of the inert gas the lung volume can be calculated   Most of the methods used for the measurement of lung volume are based on this principle, but it has only recently been appreciated that the rate at which gaseous equilibrium is reached may provide an index of lung function (7) Bateman (4) and Cournand (3) have investigated the efficiency of mixing in the lung by studying the rate of nitrogen washout in a group of patients , but this method is laborious, and it was felt that there would be several advantages in using a method based on the rate of mixing in the lungs of an inert gas such as helium

While work along these lines was in progress, Meneely and Kaltreider (5) published a study of the rate of helium mixing in the lungs and showed that in 16 cases of emphysema the mixing rate was retarded   No allowance was made for changes in lung volume or tidal air, and therefore no reliable index of mixing efficiency was obtained   In addition, the rate of mixing was plotted against time which is misleading since it is the number of complete respirations which is the important factor in determining the rate of mixing

It was to devise an index of mixing efficiency which takes into account variations in lung volume and tidal air, that the present investigation was started

Apparatus

The apparatus is based on that described by McMichael (1 and 2) for the measurement of the functional residual air, except that helium is used as the indicator gas instead of hydrogen   It has been shown recently by Gilson and Hugh-Jones (6) that this closed circuit method using helium and a katharometer is an accurate and relatively rapid method of determining

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the functional residual air, it can also be used to measure the rate at which helium is mixed with the air in the lungs, but for this purpose the circuit must be modified in certain respects   (Fig 1)

(a) *Pump*   In the apparatus described by Gilson and Hugh-Jones, the rate of circulation and mixing in the main circuit was not material as ample time could be given for equilibrium to be established before a final
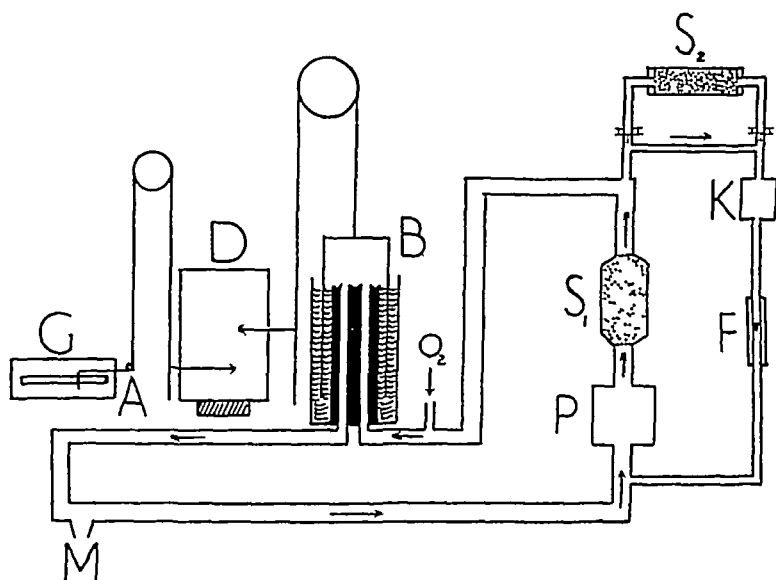
Fig 1   *Circuit diagram*   A = mechanical tracker   B = spirometer   F = flowmeter   G = galvanometer   K = katharometer   M = mouthpiece   P = pump S_1 = main circuit CO_2 scrubber S_2 = side circuit scrubber   D = recording drum.

galvanometer reading was taken, but for the plotting of a mixing curve it is desirable to have as rapid a circulation as possible in the main circuit   The pump (P, Fig 1) used is a high speed fan—similar in construction to the familiar hair dryer—with an output when in the circuit of about 50 litres/min This ensures that one complete " flush " of the main circuit occurs every seven seconds   An airtight bearing must be made between the fan blades and the commutator as the motor brushes must not be in contact with the circuit containing oxygen

(b) *Katharometer*   (K, Fig 1)   This instrument consists of two pairs of resistances, one pair sealed in pure oxygen, and the other open to the gas stream   The galvanometer records the change in potential across these when the open pair are in contact with helium   It is calibrated from 0—15% helium in oxygen over a ten-inch deflection, and is provided with a switch so that it can be made to measure the voltage output of the batteries used as the current source.

A fall in the current to the katharometer results in a considerable error and it is essential that the current should be kept constant throughout the plotting of the mixing curve It was found that standard cells of the car battery type were unsuitable and special low loss type cells* are now used These are trickle charged every night and left on open circuit during the day and can be relied upon to give a constant current during the run   The voltage output of the cells is checked immediately before and at the end of each run

It is important that the gas mixture should pass through the main CO_2 scrubber (S, Fig 1) before reaching the katharometer   The rate of flow through the katharometer (K, Fig 1) is observed by means of a bobbin flowmeter (F, Fig 1)   This must be kept below 2 litres a minute if a direct cooling effect is to be avoided, and it is normally adjusted to 1 litre a minute for each run as in this way the lag is kept fairly constant   All gases are bubbled through water into the main circuit, which contains no wash bottle   During a run condensation can be seen in the glass tube that leads to the katharometer showing that the gases are saturated with water vapour   A small CO_2 scrubber (S_2, Fig 1) is inserted in a bypass off the katharometer circuit   This is switched in at the end of a run to make sure that CO_2 absorption is complete

(c) *Temperature*   By the insertion of thermometers into the circuit it was found that the temperature of the gas beyond the CO_2 scrubber was 6°C higher than it was beyond the spirometer at the end of a run   The rise of temperature at the katharometer was only 0 5°C during the same period It is important therefore to have a sufficiently long lead to the katharometer to allow cooling, but this must be balanced against the delay in response time that a long lead must produce , in our apparatus it is just over 12″ long   A thermometer is permanently inserted beyond the spirometer and the temperature taken before and after each run

(d) *Tracker*   A mechanical tracker (A, Fig 1) is fitted to the outside of the galvanometer, and is so connected that it records directly below the spirometer tracing   The tracker is moved by a wheel fitted to the end of the galvanometer (G, Fig 1) and is counterbalanced so that movement is smooth and easy   The wheel is manually operated, the fall of the galvanometer needle being followed visually by keeping the pointer which runs along the front of the scale in line with the galvanometer needle

This has been found a most satisfactory way of recording the fall in helium percentage

(e) *Technical details*   A syphon water level indicator is inserted into the water seal of the spirometer jacket so that the level of this may be kept constant   Care must be taken that with the fan in operation, the pressure at

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* These cells were supplied by Exide Ltd , and are numbered LLZG–2

the mouthpiece is atmospheric   All tubing is 1″ in diameter, and angle pieces are made of brass   The apparatus must be tested routinely for leaks, though these have not proved troublesome   The volume of the circuit dead space with the spirometer empty is 4300 c c

Procedure

The procedure for the determination of the mixing rate and functional residual air of a patient is as follows

A tracing of O_2 uptake is taken and three vital capacities are recorded The patient is now switched to a bag containing oxygen and the circuit is flushed with oxygen   The spirometer is then set at 1000 c c on the scale so that the circuit dead space is the same for each run   The cell voltage is checked, and the needle of the galvanometer adjusted to zero by altering the rheostat resistance in the katharometer   700 c c of helium is now admitted into the circuit, and the galvanometer allowed to stabilize   The cell voltage is again checked, and the circuit temperature taken   The tracker is aligned with the galvanometer needle and the kymograph started Then, at the end of an expiration, the patient is switched from the bag into the circuit and at the same time oxygen is run in to balance the patient's oxygen consumption   The fall of the galvanometer reading is accurately followed with the tracker until mixing is complete   When this has occurred, the patient is switched from the circuit and the O_2 input switched off   The cell voltage is checked and a galvanometer reading is taken   The katharo-meter side circuit containing the extra CO_2 scrubber is then switched in and a final reading is taken

The circuit is now washed with oxygen until the galvanometer returns to zero, and the whole procedure is repeated so that two readings for the functional residual air and two mixing curves are obtained on each patient

Since the katharometer is slightly sensitive to nitrogen, any considerable change in nitrogen percentage should be avoided   Nitrogen may be washed out by breathing oxygen for ten minutes in normal subjects, and in patients with emphysema for twenty minutes

Fig 2 shows the chart obtained from a normal person by means of the technique described above

Calculation of formula

It has been calculated that the following expression relates the fall in helium percentage to the variables in the circuit provided that mixing is complete both in the main circuit and in the lungs between each respiration

The formula is as follows

*Formula " A ' ***

$$\% \text{ helium} = \frac{100 \text{ H}}{\text{V} + \text{F}} \left[1 + \frac{\text{FQ}^r}{\text{V}} \right]$$

where V = Volume of main circuit at the beginning of inspiration

H = Initial volume of helium

F = F R A

T = Tidal air

r = number of complete respirations

and $Q = \dfrac{\text{F(V—T)}}{\text{V(F+T)}}$

From this expression, the following formula can be derived which predicts the number of respirations required to achieve 90% mixing

Formula   B

Number of complete respirations to achieve 90% mixing $= \dfrac{-1}{\log O}$

Experiments with model lung

A model lung was constructed so that formula " A " described above could be tested   The model consisted of a spirometer with a small dead space, and a pump circulating the air at a rapid rate   This circuit could then be filled with oxygen and be switched into the main circuit in the same way as a patient, except that the two circuits could be disconnected after each " breath," so that complete mixing in both the main circuit and the model lung could be ensured   In this way, the two assumptions of the formula could be fulfilled, and it would be possible to check its accuracy

Five experiments on these lines were done with different values for the model volume and the tidal air in each case   The agreement between the actual and predicted curves was very close, the average deviation of the differences being in no case greater than 0 16% helium   It was therefore concluded that the formula was a correct expression for the fall in helium percentage

Fig 3 shows a number of theoretically calculated mixing curves, and it will be seen that variations in the functional residual air and tidal air produce considerable differences in the shape of the curve   This illustrates well that an estimate of mixing efficiency without taking into account changes in the functional residual air and tidal air is bound to be fallacious

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* *See* Appendix for details of calculation

It was clearly necessary to have a simple means of expressing the difference between the plotted curve of helium fall in a patient and that calculated from the formula   It was decided that the following ratio would express any delay in mixing in a convenient way

$$\frac{\text{Theoretical No of respirations to reach } 90\% \text{ mixing}}{\text{Actual No of respirations to reach } 90\% \text{ mixing}} \times 100$$

Experiments with the model operated at different respiratory rates showed that with a respiratory rate of 1/min this ratio was 100, but that due to delay in main circuit mixing and katharometer response, it fell to 80 at rates between 1/min and 8/min , and thereafter remained steady at 80 at rates between 8/min and 25/min   Since it can be assumed that mixing in the model was complete up to rates of 25/min , it follows that within the physiological range of respiratory rates, individuals with perfect mixing in the lungs should give a ratio of 80   That it is not 100 is due to delays in the main circuit mentioned above, and for this reason, when dealing with patients and normal individuals the ratio was scaled up from 80 to 100 Thus a patient with a ratio of 60% for example, is said to have a mixing efficiency of 75%

THEORETICAL CURVES

	FRA	TA	90% MIX	
A	= 3,300	450	11	RESPNS
B	= 4 510	350	19	"
C	= 4,470	698	10	"
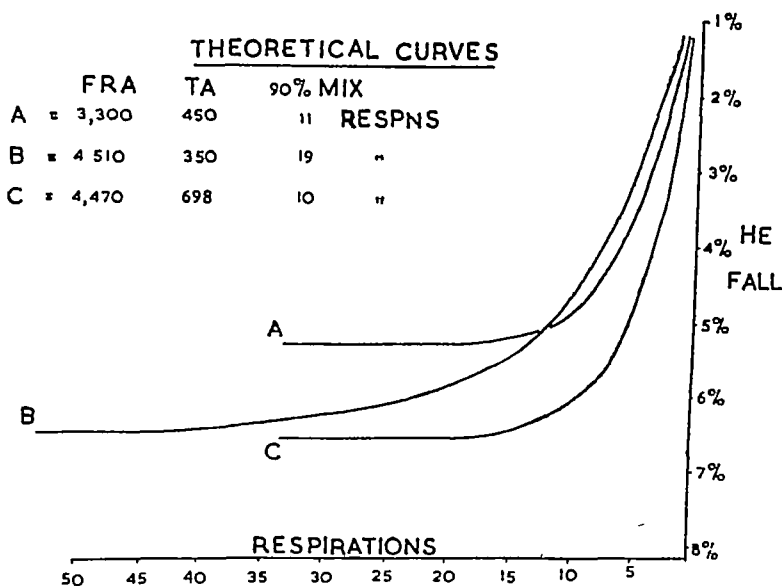
Fig 3   *Theoretical curves*   Curves showing the predicted rate of fall in helium percentage plotted against the number of respirations, for different lung volumes and tidal air values Perfect mixing in the lungs is assumed   Changes in lung volume and tidal air produce considerable differences in mixing rate even when mixing is perfect

Several experiments were done to investigate the relationship between tidal air and volume of the model lung on the mixing rate, and it was found that for volumes between 2 and 5 litres and for tidal air variations from

300 c c to 900 c c, the mixing efficiency varied by only 8% on different occasions   The mixing efficiency of the model could be lowered by slowing the pump in the model, and in the extreme case when the model pump was switched off so that mixing was by turbulence and diffusion only, the mixing efficiency dropped to 24%   This is of interest as this is the sort of figure obtained in emphysema

A further experiment was performed in which a length of tubing of 170 c c volume was interposed between the two circuits, and isolated from them by a tap at each end after each breath   This simulated an increase in the respiratory dead space, and the effect of it was to lower the mixing efficiency from 100% to 81%   The significance of this experiment is discussed later

TABLE I
Data from a group of 47 subjects

Group		Age	Tidal air c c	Respirations per minute	Functional residual air (F R A) in litres	Residual air expressed as %age of total lung volume	Mixing efficiency % (M E %)
Young Normal	Range	17 to 37	420 c c to 1100 c c	7 to 21	2 39 l to 4 60 l	21% to 42%	62% to 98%
17 subjects	Mean	27	690 c c	17	3 15 l	26%	76%
Old Normal	Range	47 to 62	520 c c to 1400 c c	8 to 26	2 16 l to 3 91 l	29% to 46%	32% to 86%
10 subjects	Mean	53	936 c c	19	3 16 l	37%	54%
Emphysema	Range	38 to 65	250 c c to 860 c c	8 to 35	2 80 l to 6 17 l	52% to 78%	11 5 % to 37%
20 subjects	Mean	56	535 c c	21	4 63 l	66%	25%

RESULTS

In emphysema the mixing efficiency is clearly impaired (Fig 4)

Table I summarises the data obtained in a series of 47 subjects   The difference in mixing efficiency percentages is also shown diagrammatically in Fig 5

Three principal groups of patients were studied   The young normal group (Ny), had no history of chest disease and were normal clinically   The old normal group (No), with an average age of 53, had no history of chest disorder, were normal clinically and radiologically, and had no exertional dyspnœa   The emphysema group (E), were patients with clinical emphysema and exertional dyspnœa   This group includes patients with and without co-existent bronchospasm, and includes three patients with chronic
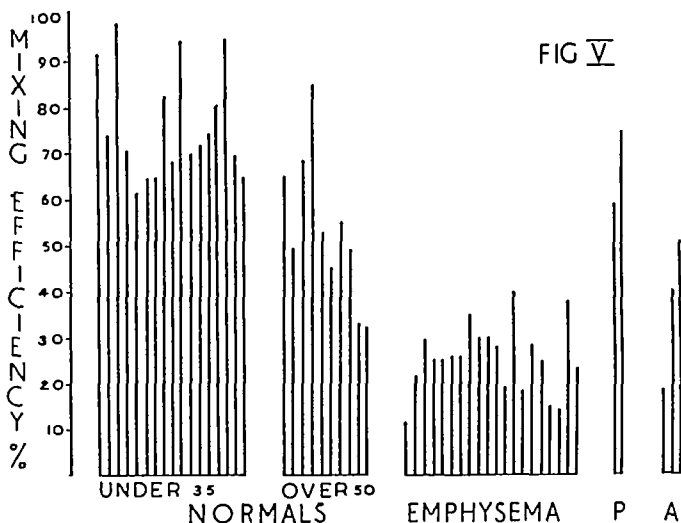
Fig 5   *Mixing efficiency*   The mixing efficiency (ME%) is shown as the ordinate   P = spontaneous pneumothorax collapsed and after re-expansion   A = asthmatic with progressive recovery

cor pulmonale   These three had the lowest mixing efficiency of the group , the rest were ambulant and the majority still at work   It can be seen that the average mixing efficiency percentages of the three groups was 76%, 54%, and 25% respectively   The differences of 22% between the young normal and the old normal groups is a statistically significant difference

Two cases have been investigated in addition to the above groups   The first (P, Fig 5) was a lad, 18 years of age, with a non-tuberculous spontaneous pneumothorax on the left side   At the time of the first estimation the left lung was about half collapsed   The second estimation was done when re-expansion was complete and it will be seen that there is a difference of 16% in the mixing efficiency on the two occasions

The second case (A, Fig 5) was a patient with severe bronchial asthma   This patient, aged 42, had a three-month history only of asthma, but had been disabled by a continuous and severe attack for the past two months   He had been bedridden for most of this period and was resistant to adrenalin in doses of up to 60 minims   On the first occasion that the estimation was done he was in a severe attack and the mixing efficiency percentage was 18%   He was much improved clinically by the time the second estimation

was performed, and his mixing efficiency percentage had risen to 40% A final estimation a week later showed that it had risen again to 51%

Discussion

There has in the past been considerable confusion of thought concerning the role of the dead space of the lung in intrapulmonary mixing This has arisen because the term dead space has been used in two senses, firstly to describe the space in the trachea and bronchi which is not concerned with hæmo-respiratory exchange, and secondly to describe parts of the lung which are poorly ventilated To use the term dead space for both of these is clearly misleading since the trachea although not in contact with the pulmonary circulation is well ventilated, while the underventilated areas in the lung may be in intimate contact with pulmonary blood

We consider that the rate of inert gas mixing is influenced by two factors

1 The effect of the trachea and larger bronchi (anatomical dead space) in preventing a proportion of the inspired air from coming into contact with alveolar air

2 Unequal ventilation in the lungs

It seems probable that in normal individuals the influence of the first of these factors is sufficient to prevent mixing from being 100% perfect, and it will be seen from our results that the average mixing efficiency of the young normal group was 76% We have preferred not to make any correction for the anatomical dead space, since to do so would be to introduce an arbitrary figure the accuracy of which in health and disease is unknown

In emphysema the impairment in mixing efficiency is much greater than can be accounted for by the comparatively slight increase in anatomical dead space which has been shown to occur in this disease The experiment with the model lung described above showed that an increase in dead space of 170 c c, lowered the mixing efficiency by only 19%, whereas there is a difference of 50% between the normal and emphysema groups It seems unlikely therefore that an increase in anatomical dead space is a significant factor in impairing mixing in emphysema, though one cannot say for certain that it plays no part The weight of evidence is strongly in favour of accepting the second factor, unequal ventilation, as mainly responsible for the impairment in mixing that has been demonstrated

It is not possible to state precisely what proportion of the lung is underventilated in any given case, and we have felt therefore that an expression based solely on the actual delay in mixing is, for the moment, the most satisfactory method of measuring mixing efficiency

Summary

1 A method of measuring the mixing efficiency of the lungs is described By means of a formula, it is possible to eliminate the effect of changes in lung volume and tidal air on the mixing rate

2   The mixing efficiency is severely impaired in emphysema, and was reduced in one case of pneumothorax   In a case of severe asthma, recovery was accompanied by a progressive increase in mixing efficiency

3   The significance of these findings is discussed

APPENDIX

Derivation of formulae

Let V   = Volume of main circuit at the beginning of inspiration

H   = Initial volume of helium

F   = F R A

T   = Tidal air

r   = number of complete respirations

$$Q = \frac{F\,(V-T)}{V\,(F+T)}$$

Let the patient begin by breathing in

On the assumption that mixing is complete both in the main circuit and in the lungs between each inspiration and expiration, consider the change in helium in the main circuit

No of respirations	*Helium in main circuit*	*Helium in lungs*
Initially	H	Nil
1 Inspiration	Ha	$H\,(1-a)$
Expiration	$H\,(1-b+ab)$	$H\,(b-ab)$
2 Inspiration	$H\,(a-ab+a^2b)$	$H\,(1-a+ab-a^2b)$
Expiration	$H\,(1-b+ab-ab^2+a^2b^2)$	$H\,(b-ab+ab^2-a^2b^2)$
rth Inspiration		
Expiration	$H\,\left[(1-b)\,(1+ab+a^2b^2+ \quad +a^{r-1}b^{r-1})+a^rb^r\right]$	

where $a = \dfrac{V-T}{V}$ and $b = \dfrac{F}{F+T}$ and hence $Q = ab$

Summing the geometric progression above, it follows that the volume of helium left in the main circuit after the rth complete respiration is

$$\frac{HV}{V+F}\left[1+\frac{FQ^r}{V}\right]$$

and the % helium after the rth complete respiration is

$$\frac{100\,H}{V+F}\left[1+\frac{FQ^r}{V}\right]\qquad\text{FORMULA ``A''}$$

From this, the number of respirations to produce 90% mixing is

$$\frac{-1}{\log Q}\qquad\text{FORMULA ``B''}$$

These formulæ were developed by J A Heady, statistician to the Hospital, and Mr F Meade of the M R C Pneumokoniosis Research Unit at Cardiff

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INTRAPULMONARY MIXING
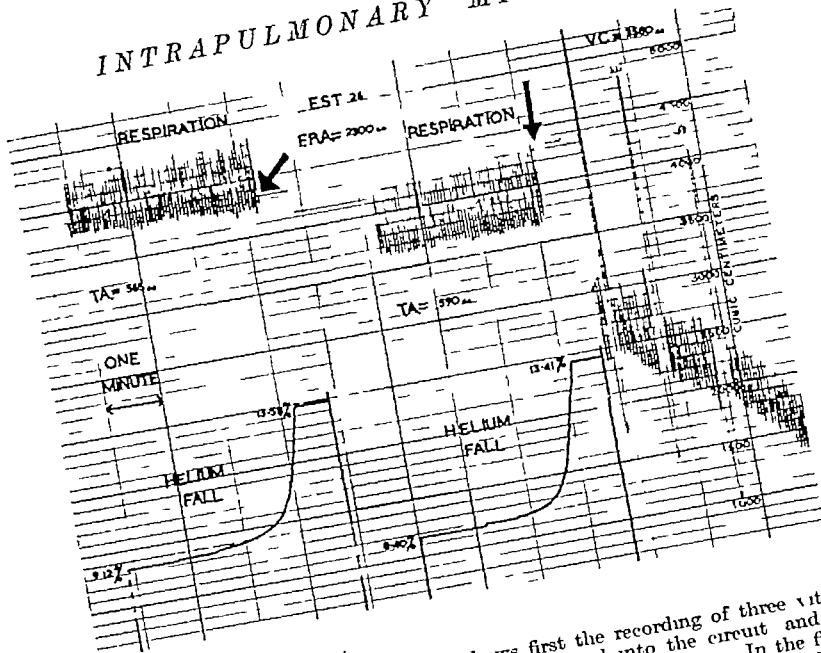
Fig 2 *Record from normal subject*
The record reads from right to left and shows first the recording of three vital capacity measurements At the arrows the subject is switched into the circuit and the fall in helium percentage can be seen plotted below the respiratory tracing In the first run the subject was switched in at the beginning of expiration and in the second at the beginning of inspiration This accounts for the difference in the final helium percentage in the two runs Mixing in this subject was complete in about 30 respirations (2 minutes) VC = vital capacity TA = tidal air FRA = functional residual air
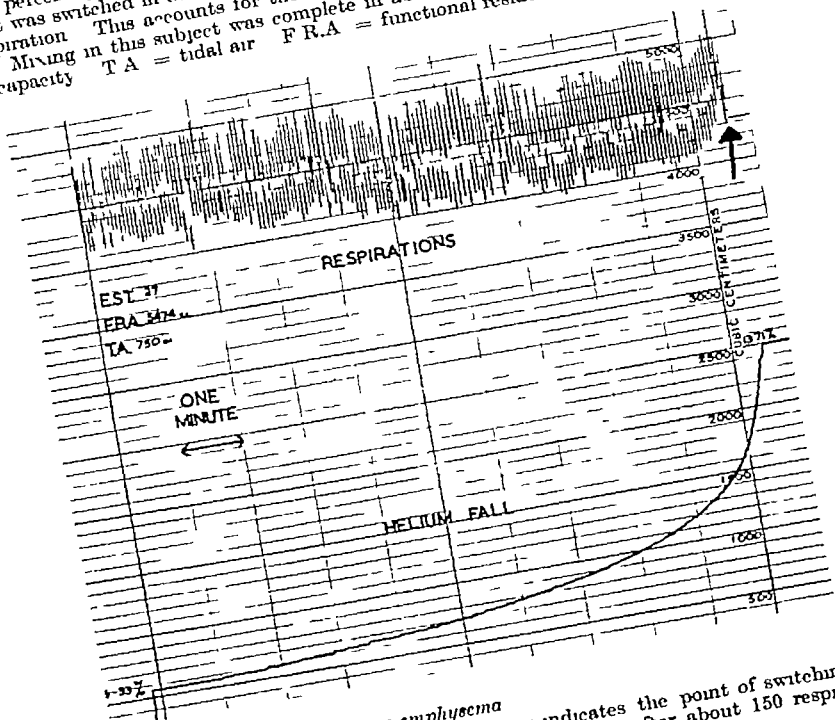
Fig 4 *Record from patient with severe emphysema*
The record reads from right to left The arrow indicates the point of switching into the circuit It will be seen that mixing was only complete after about 150 respirations (10 minutes) Compare with Fig 2

VASODILATATION IN THE HAND IN RESPONSE TO HEATING THE SKIN ELSEWHERE

By D McK KERSLAKE and K E COOPER *

(From the R A F Institute of Aviation Medicine, Farnborough, Hants)

THE application of moderate heat to small areas of skin is known to give rise to generalised peripheral vasodilatation (1, 5, 9, 16, 19, 20, 23, 24)   Pickering (19) heated the forearm and hand in water at about 42°C and found that vasodilatation occurred in the opposite hand   The response was absent when the circulation to the heated limb was arrested, and appeared when the occlusion was released   The author concluded that the afferent stimulus causing vasodilatation was an increase in blood temperature and did not derive from sensory endings in the skin

Observations supporting this hypothesis were made by Uprus, Gaylor and Carmichael (23), who measured the rectal temperatures and skin temperatures of the hands of subjects whose feet were placed in warm water The rise in skin temperature was found usually to be coincident with a rise in rectal temperature, occurring after a delay of some minutes, although in some cases the rectal temperature was falling while vasodilatation took place The latter observation was frequently made when heating was carried out by applying a hot air bath to the trunk   No explanation for the finding was offered, and the authors concluded that the results upheld Pickering's hypothesis   On the other hand, the fall in rectal temperature might be expected if the vasodilatation were due to afferent nervous impulses from the heated area ,   an increased blood flow through the relatively cool limbs might result initially in a loss of heat to these regions greater than the absorption of heat from the warm air or water

Generalised reflex vasoconstriction in response to local cooling was first described by François-Franck (8) in 1876   It has since been fully confirmed by other workers (19, 20, 22)   The nature of the constrictor response to cold was examined by Pickering, who found that vasoconstriction in the hand, as estimated by the Stewart calorimeter, occurred when the opposite hand and forearm were cooled with their circulation arrested (19) The constriction so caused was only transient, and the heat elimination of

the control hand returned to normal after a few minutes   When the circulation was then released, a second, lasting, vasoconstriction was observed in the control hand, and this was associated with a fall in rectal temperature   The mechanism of the constrictor response appeared therefore to be twofold, and the operation of the two components can be seen in records of the response to cooling the unoccluded limb (22)

It is natural that analogous responses to heat should be sought, but, so far, conclusive evidence has been obtained only in the case of vasodilatation in response to a rise in blood temperature (9, 19)   Some results suggesting that afferent nervous impulses from the heated area may, under certain circumstances, produce generalised vasodilatation have been reported, and are reviewed by Duthie and Mackay, who themselves present further evidence (5)

The heating of quite small areas of skin by infra-red light was found by Bader and Macht (1) to cause a rise of skin temperature of the hand   The delay in onset led these authors to conclude that with this form of heating afferent nervous stimuli did not initiate the response   The skin temperature is not a good index of rapid changes in blood flow unless the ambient temperature is low (6) and it is possible that these results could be explained satisfactorily in other ways

The results to be presented indicate that when larger skin areas are used, vasodilatation in the hands is dependent at first upon the stimulation of nerve endings in the skin

Methods

The experiments were performed in a warm room at 20-28°C   Air movement was kept to a minimum, and, apart from the sounds produced by the apparatus, silence was maintained

The investigations were carried out upon healthy young male members of the laboratory staff, most of whom had previously been used in similar experiments   The subject, wearing bathing trunks only, lay on his back on a stretcher, his head supported on a low pillow   Heat was applied, either to the front of the trunk or to the front of the legs, by means of a radiant heat cradle   The cradle consisted of a cylindrically curved aluminium reflector three feet long, containing six 100 watt household electric light bulbs   The ends were partly closed in by metal   The heat dissipation of the lamps could be reduced from 700 to 350 watts by introducing a series resistance into the supply lead   The area exposed to radiation when the front of the trunk was heated extended approximately from the clavicles to the knees   The cradle was placed with its upper end level with the fourth ribs in front, so that the left arm could be conveniently placed for insertion into the plethysmograph   When the legs only were heated, the upper end of the cradle was level with the middle of the femurs, and the radiation was carefully screened from the abdomen

Blood flow in the left hand was measured by means of a water filled venous occlusion plethysmograph The plethysmograph was maintained at 30°C and the water in the interior was circulated The collecting cuff was inflated to 60 mm Hg at regular intervals automatically (13) The elbow and shoulder were supported on air cushions, so arranged as to ensure that no compression of nerves or vessels could occur The arm was not itself exposed to the radiation The final position of the apparatus was adjusted under the subject's instructions, so that he might be as comfortable as possible

In some experiments the circulation to the legs was arrested by means of large sphygmomanometer cuffs (18 in by 6 in) applied to the upper part of the thigh and inflated to 200 mm Hg pressure   -

Efforts were made to ensure that the subjects were as comfortable as possible Except in the experiments involving occlusion of the circulation to the legs, the subjects had no complaints, and all either dozed or fell asleep The constriction of the thighs was unpleasant, but not intolerably painful Little change in resting blood flow was observed when the cuffs were inflated, and it was concluded that the discomfort did not interfere seriously with the circulatory responses

The method of interpretation of the blood flow records is of some importance, and was carried out in the following way A pencil line was drawn parallel to the inflow curve, or to the early part of this if the whole curve was not straight The time marker produces vertical lines on the record at preset intervals, so that the vertical distance between the points at which the pencil line crosses the first and last time marks in the record is a simple function of the blood flow This distance was measured in arbitrary units by placing on the record a mask made by photographing a graph paper lattice onto a lantern slide The corner of the lattice was placed over the first inter-section, and the horizontal lines on the mask brought parallel with the reference lines on the record The point of intersection, of the pencil line with the last time mark was then read off from the lattice, whose abscissæ were ignored The value obtained in this way could be converted to absolute units by multiplying by a factor derived from the hand volume, the dynamic calibration of the volume recorder and the time interval between the first and last time marks on the record Individual flows were, however, usually expressed in arbitrary units, the conversion to absolute units being carried out only upon the averaged results of groups of flows Apart from the saving of labour, this method omits one step of approximation

It will be noticed that the estimate of blood flow is based on the absolute rate of increase of hand volume during the first few seconds after the collecting cuff is inflated It takes no account of the possible trends in resting hand volume immediately preceding the application of the collecting

pressure   It was felt that if the conditions of venous occlusion plethysmography are fulfilled, namely, that the collecting pressure initially prevents all venous return but does not affect arterial inflow, then the blood flow is represented by the absolute rate of change of hand volume, and the inclusion of the changes in resting hand volume is neither desirable nor justifiable

Design of experiments   When blood flow changes were observed over periods of 20 min , readings were taken in groups of three every minute, each group occupying a total of thirty seconds, and the average of the three inflows was taken as the blood flow for the minute concerned

In those experiments in which changes of blood flow during the first minute of heating were being examined it was not possible to average consecutive readings in this way, since the number of accurate readings obtainable could not exceed seven per minute   A minimum of four seconds of application of the collecting pressure is needed in order accurately to compute the slope of the inflow curve, and an equal period must be allowed between readings so that the venous congestion may subside   The influence on the results of spontaneous fluctuations in blood flow, which may be large compared with the effects under investigation, was diminished by repeating the experiment a number of times and taking the average of the blood flows observed at corresponding times in the several experiments   Since the blood flow was found to return to its resting level within 30 sec of switching off the lights, it was considered that the repetition of experiments at intervals of four minutes was justifiable

<div align="center">RESULTS</div>

Prolonged heating of the front of the trunk   After allowing sufficient time for the hand blood flow to stabilize, the heat cradle was turned on and observations of blood flow were continued for 15 min   Typical results are shown in Fig 1   It will be seen that there is a rapid increase in blood flow reaching a maximum after about 5 min and maintaining this level for the remainder of the period of observation   The vasodilatation is well established by the end of one minute of heating

Changes during the first minute of heating   In order to determine the time of onset of the vasodilatation more exactly, arrangements were made for inflow curves to be recorded at intervals of 8 sec   (In other experiments this was increased to 10 or 12 sec)   After recording five such inflow curves the lights were turned on and recordings continued   The lights were turned off after five readings, and a final group of five readings was recorded   The experiment thus consisted of fifteen consecutive readings   The cycle of application and release of the collecting pressure was continued, although records were not taken, and after two minutes the whole procedure was repeated   A series of experiments was performed at each session, the number being limited by the tolerance of the subject or the length of recording paper in the camera   The records from the set of experiments were analysed and

arranged as in Table I   The results were then averaged and converted to
c c per 100 c c hand per min   The results of the experiments in Table I
and from two other sets of experiments carried out on the same subject on
the same day, but at different room temperatures, are shown in Fig 2
In two of the sets the lights were turned on at the end of the fifth inflow and
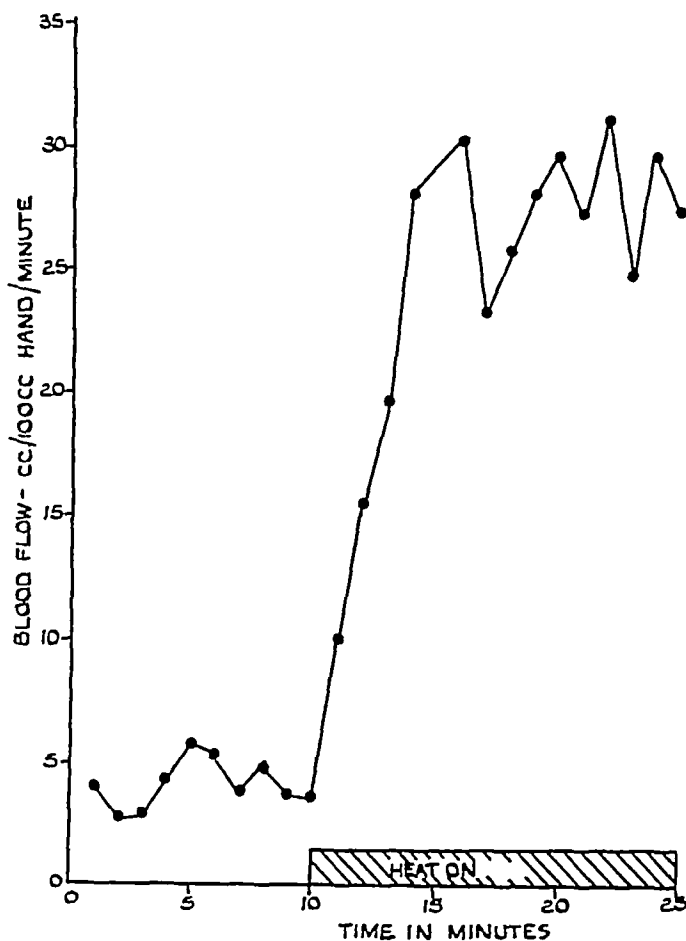
Fig 1   Changes in hand blood flow during prolonged heating of the front of the trunk.   Each
point represents the mean of three inflow curves

off at the end of the tenth   In the third (resting blood flow about 20 c c /100
c c /min) the lights were turned on at the beginning of the sixth reading and
off at the beginning of the eleventh   It will be seen that in the lower curves
dilatation begins between six and fourteen seconds after the beginning of
heating   The seventh reading in the upper curve falls during this interval

and does not show evidence of vasodilatation, which can therefore be supposed to begin approximately a quarter of a minute after the lights are turned on In some experiments the inflow curves taken at about this time showed a greater slope at the end than the beginning, and the onset of vasodilatation could be seen in the record   As, however, such changes are occasionally seen in resting inflow curves, it is not possible to assign significance to any single observation of this type

TABLE I

Results of 8 similar experiments, in which blood flows were recorded at 8 sec intervals, on subject D McK K   Individual inflows are expressed in arbitrary units, the means being converted to c c /100 c c /hand/min   Lights turned on at end of the 5th reading and off at the end of the 10th reading

Hand blood flows

Reading	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Average c c/100 c c /min
1	11	15	13	10	7	9	12	8	7 1
2	14	14	11	10	5	9	11	6	6 7
3	18	13	11	12	10	7	13	7	7 6
4	18	12	14	16	7	6	12	8	7 7
5	21	17	11	13	10	9	14	10	8 7
6	12	10	12	16	11	8	11	7	7 2
7	23	21	18	28	16	19	19	17	13 4
8	23	22	25	30	26	21	23	19	15 7
9	30	28	35	36	34	29	32	25	20 7
10	30	33	31	32	30	34	32	34	21 4
11	27	36	22	25	33	25	34	31	19 4
12	21	15	8	13	16	8	12	10	8 6
13	18	14	12	18	12	9	14	15	10 2
14	16	11	12	16	13	11	12	11	8 5
15	18	17	13	11	10	10	14	8	8 4

The responses of all the other subjects studied were essentially similar, although the extent of the vasodilatation was not always so great   A few subjects showed, particularly in the first few experiments on them, an initial constriction, the first observation after the lights were turned on being markedly lower than the resting

A rough estimate of the extent of the vasodilatation in any group of experiments can be made by averaging the five resting blood flows and subtracting this amount from the average of the five flows observed during the period of heating   The initial constriction in some of the subjects, which is probably due to the surprise occasioned by the sudden change in sensation

from the skin, will cause the results in these cases to be rather lower than the true values, but it has been thought best to include all the observations taken during the period of heating, and not to try artificially to eliminate this factor
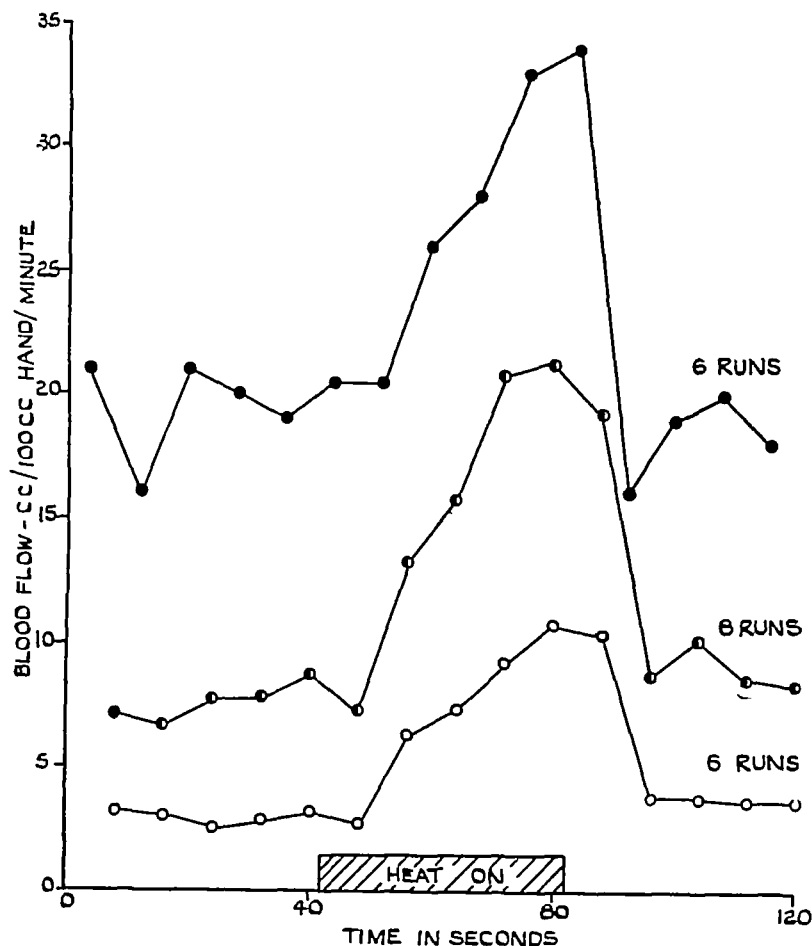
Fig 2   Changes in hand blood flow during 40 sec of heating the front of the trunk.   Three curves obtained at different room temperatures on the same day   Upper curve, 6 experiments at room temperature 28°C , middle curve 8 experiments at 24°C , lower curve, 6 experiments at 20°C

The averaged results obtained at each experimental session for the six subjects studied are set out in Table II, and the mean increase in blood flow during the period of heating, calculated as described in the previous paragraph, is given in each case   It will be seen that on every occasion, except one, the mean second reading after the lights are turned on is greater

TABLE II

Changes in hand blood flow during the first minute of heating the front of the trunk   Lights turned on at the end of the fifth reading and off at the end of the tenth

Subject	Date	No of experiments	Rate of heating	Period of heating (sec)	Readings per min	Mean blood flow—c c /100 c c /min															Mean dilatation
						Reading number															
						Resting					Heating					Resting					
						1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
K E C	0 9 48	5	Full	00	5	52	54	51	50	45	37	68	84	89	04	45	67	52	57	52	24
	0 4 49	4	Full	50	6	20	15	19	17	10	19	34	43	41	43	30	32	21	21	17	36
	0 4 49	4	Half	50	6	15	15	12	18	15	14	27	21	21	32	35	18	21	21	17	08
D R W	5 10 48	3	Full	00	5	54	53	55	45	70	31	85	120	140	102	69	90	79	66	50	40
	7 10 48	10	Full	60	5	50	52	58	59	41	42	104	137	154	151	52	67	63	71	53	06
A J B	1 11 48	6	Full	60	5	86	91	97	74	03	49	130	145	142	183	52	120	100	74	70	42
M B	2 11 48	5	Full	00	5	30	26	30	37	30	29	71	80	95	103	31	01	41	44	40	45
W K S	11 10 48	4	Full	00	5	17	20	21	16	20	23	35	44	39	44	21	18	20	19	23	18
D McK K	1 10 48	6	Full	40	7 5	21	16	21	20	19	21	21	20	28	33	34	16	19	20	18	64*
	1 10 48	8	Full	40	7 5	71	67	76	77	87	72	134	157	207	214	104	86	102	85	84	81
	1 10 48	8	Full	40	7 5	32	30	25	29	32	27	61	73	03	107	103	40	38	36	36	43
	6 9 48	3	Full	00	5	58	62	60	71	62	88	134	104	197	223	70	50	54	62	05	99
	6 9 48	3	Half	00	6	37	41	38	31	35	40	65	94	95	110	36	45	45	47	41	46
	20 5 49	4	Full	60	6	24	27	24	26	12	20	49	68	71	76	11	17	13	13	12	34
	20 5 49	4	Half	50	6	19	13	14	16	17	17	33	37	46	45	19	17	16	09	13	20

* Lights turned on at beginning of sixth reading and off at beginning of eleventh

than the greatest mean flow observed during the resting period, so that it is reasonable to suppose that vasodilatation is established at or before the beginning of this inflow curve   The time interval varies from 12 sec (at 7 5 readings per minute) to 18 sec (at 5 readings per minute)   The exception to this, marked with an asterisk, is the series already referred to and shown
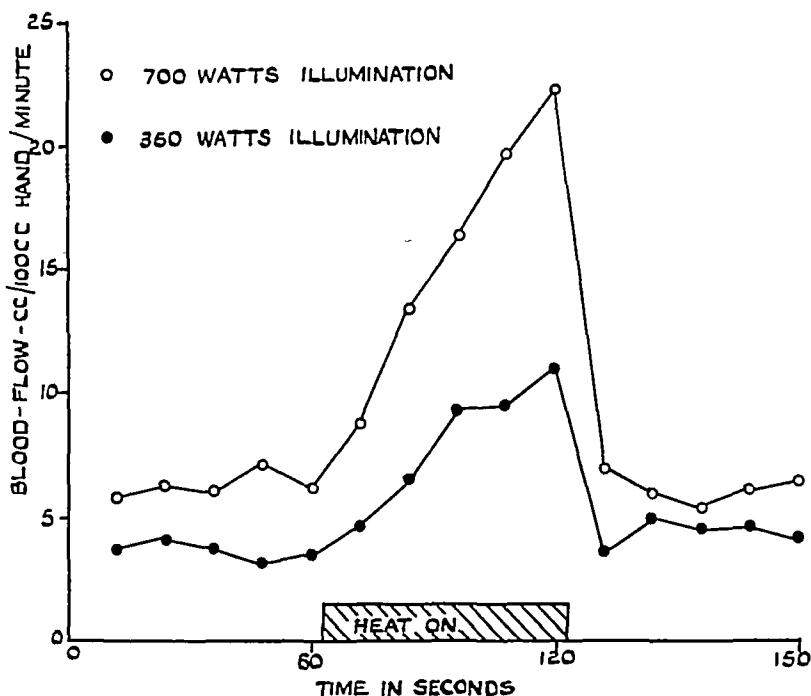
Fig 3   The effect of different rates of heating of the front of the trunk on the hand blood flow during one minute of heating   Upper curve 700 watts, lower curve 350 watts   Each curve is the mean of three runs

in Fig 2, where the lights were turned on at the beginning of the sixth reading instead of at the end of the fifth   The second reading during the period of heating in this series began only 8 sec after the lights were turned on   In only two cases does the first reading during the period of heating show evidence of vasodilatation (D McK K , 6 9 48)   The figures are in these cases greater than the greatest resting flow, but such results would be expected to occur occasionally by chance and cannot be regarded as proving that the dilatation had begun within 9 sec of the beginning of heating   The fact that both these observations were made during the same experimental session (the high and low illumination runs were alternated) does, however, lend some support to the suggestion that the figures are not entirely without significance

To summarise , the results of the experiments carried out at a rate of 5 readings per minute place the onset of the vasodilatation between 12 and 18 sec after the lights are turned on , 6 readings per minute, between 10 and 15 sec , and 7 5 readings per minute (including one staggered series at a high resting flow), between 10 and 13 sec A figure of about 13 sec would be compatible with all these results

The experiments carried out at reduced illumination show no evidence of a difference in time relations of the response, although the extent is markedly reduced The results of a series of experiments using alternately half and full illumination are shown in Fig 3 The other two series, performed in the same way (K E C , 6 4 49, and D McK K 20 5 49) give similar results

TABLE III

Subject	Date	Time of onset of vasodilatation	
		High illumination	Low illumination
K E C	11 10 48	9 6 seconds	9 6 seconds
,,	,,	15 6 ,,	12 0 ,,
,,	,,	13 2 ,,	— ,,
,,	,,	12 0 ,,	12 0 ,,
,,	,,	— ,,	11 6 ,
D McK K	8 10 48	12 6 ,,	12 6 ,,
,,	,,	13 2 ,,	— ,,
,,	,,	— ,,	11 4 ,,
,,	,	12 0 ,,	— ,,
,,	,,	13 2 ,,	12 0 ,,
A J B	1 11 48	11 0 ,,	9 8 ,,
,,	,,	11 5 ,,	10 5 ,,
		Mean 12 39 ,,	11 50 ,
W.K S	11 10 48	12 0 ,,	— ,,
,,	,,	10 8 ,,	— ,,
,,	,,	12 0 ,,	— ,,

An attempt was made to demonstrate the time of onset of the vasodilatation directly by means of the long inflow technique of Grant and Pearson (10) Under conditions of low resting hand flow, the inflow curve remains straight for as long as twenty seconds before overfilling of the venous reservoir causes it to flatten   After a control inflow curve of this type had been obtained, similar curves were recorded during the first twenty seconds of heating, both at full and reduced rates   Examples of the type of record obtained
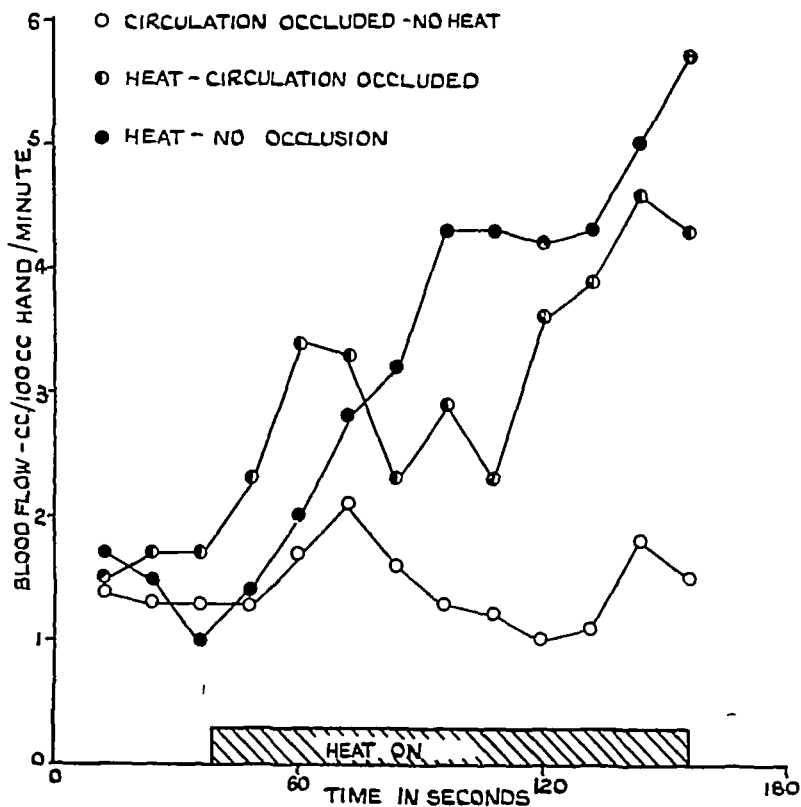
Fig 4   Changes in hand blood flow during heating of the front of the legs   Each curve is the mean of three runs

are shown in Plate 1   The onset of dilatation is abrupt and can be timed to within about a second   These records are not easy to obtain, since it is important that the blood flow must be both stable and low   A series of results obtained on different subjects is shown in Table III   There is no significant difference between the mean times of onset at high and low illumination, or between the times of onset in different subjects

TABLE IV

Changes in hand blood flow during heating of the front of the legs   Lights turned on at the end of the third reading, and off at the end of the experiment

Mean blood flow—c c /100 c c /mn

Subject	Date	No of experiments	Readings per mn	Type of experiment	Resting 1	Resting 2	Resting 3	Heating 4	5	6	7	8	9	10	11	12	13
K E C	20 9 48	2	5	C	25	22	22	25	33	37	24	20	26	30	35	28	20
		2	5	H	18	17	13	17	25	25	37	43	45	60	46	46	48
		2	5	H+C	20	22	23	21	33	35	40	44	41	44	44	52	61
K E C	4 10 48	4	7 5	C	14	17	19	19	26	26	27	26	22	23	—	—	—
		4	7 5	H	27	30	33	25	30	31	43	48	58	59	—	—	—
		4	7 5	H+C	18	15	19	19	22	31	37	40	44	56	62	53	61
D McK K	30 9 48	3	5	C	06	78	53	78	78	78	74	82	—	—	—	—	—
		3	5	H	78	60	62	60	70	98	107	132	—	—	—	—	—
		3	5	H+C	70	90	90	94	132	132	135	164	—	—	—	—	—
D.McK K	8 10 48	2	5	C	103	121	121	60	89	90	105	132	147	120	140	118	150
		2	5	H	47	42	34	58	71	62	88	110	158	184	156	174	180
		2	5	H+C	130	144	138	120	167	180	211	274	208	200	278	202	350
D.R W	24 11 48	3	5	C	14	13	13	13	17	21	10	13	12	10	11	18	15
		3	5	H	17	15	10	14	20	28	32	43	43	42	43	50	57
		3	5	H+C	15	17	17	23	34	33	27	20	27	36	30	40	43
W K S	22 10 48	3	5	C	48	52	48	58	51	62	56	37	34	38	42	40	53
		3	5	H	28	38	33	30	47	50	48	67	30	37	47	33	62
		3	5	H+C	42	53	33	33	48	40	50	63	53	69	02	58	07

Types of experiment   C, cuffs inflated, no heating,
H+C, heating with the cuffs inflated
H, heating without inflation of cuffs

Heating the legs In this series of experiments the heat cradle was arranged over the legs and the radiation carefully screened from the trunk Sphygmomanometer cuffs were applied round the tops of the thighs Experiments were divided into three groups those in which the cuffs were inflated to 200 mm Hg but no heat was applied, those in which heat was applied, but the cuffs were not inflated, and those in which heating was carried out with the cuffs inflated The heating experiments consisted of a short resting period followed by a period of heating of up to two minutes The control experiments were of equivalent length without heating The cuffs were inflated 60 sec before the beginning of the experiments in which they were used Results were averaged in the same way as those shown in Table I Typical results are shown in Fig 4, which are essentially similar to those obtained on two other subjects A fourth subject, W K S, who gave a very small dilatation on exposure of the trunk to full illumination (*see* Table II) showed very little dilatation when the legs were heated Calculation of the correlation coefficient for the mean blood flows during the period of heating with the cuffs inflated gives a value of 0 78, and a value of p of less than 0 02 A definite increase in blood flow during the period of heating is thus demonstrated In no subject is there evidence that the occlusion of the circulation to the legs affected the vasodilator response to heating them The mean results of all the series of experiments are given in Table IV

Discussion

When areas of the skin are heated as described in this paper, it seems probable that the vasodilatation occurring in the hand results reflexly from changes in the temperature receptors in the heated skin

This conclusion is strongly suggested by the short latent periods between the start of heating and the onset of vasodilatation, and between the cessation of heating and the return of the hand blood flow to its resting level Although it might be considered that warm blood from the heated skin of the trunk might reach the central heat regulating mechanism within a quarter of a minute of the start of heating, the delay in response to the termination of heating is not more than six seconds The circulation time from the lung to the tongue is known to be of the order of ten seconds, so that the peripheral circulatory responses cannot be explained on the basis of changes in the temperature of the blood going to the head

The inference that the initiation of the vasodilatation in the hand is due to nervous impulses in the heated area is confirmed by the results of the experiments in which vasodilatation was obtained when the legs were heated with their circulation arrested

As has been pointed out in the introduction, if the rate of heat loss, consequent upon the vasodilatation in the unheated areas, exceeds the rate of heat absorption from the heated area, there will be a fall in the mean

temperature of the blood reaching the right heart, and probably, therefore, in the aortic temperature   It is not considered that either the mouth or rectal temperature forms a good index of aortic temperature (3), but measurements of both these quantities and of the temperature of the œsophagus at the level of the aortic arch have been made during the first ten minutes of heating

In nearly all cases (65 out of 66 mouth temperatures, 11 out of 12 rectal temperatures, and one œsophageal temperature) a fall of temperature has been observed, regardless of the direction of the resting temperature trend   This conforms with the observations of some other workers on body temperature changes immediately following a rise in environmental temperature (4, 7, 11, 14, 15, 17, 21, 23, 24, 25)

The form of heating which has been employed may alter the pattern of afferent impulses from the skin in two ways , either direct stimulation of nerve endings sensitive to warmth may take place, or the activity of cold receptors, normally stimulated by the room air, may be reduced   The latency, of about four seconds, in the reduction of the hand blood flow when heating is stopped is comparable with that described by François-Franck (8) for the cold constrictor reflex   For this reason, it might be supposed that cold receptors in the skin become stimulated at this time   There are, however, certain objections to this view   Pickering (19) described fatigue of the cold constrictor reflex, showing that remote vasoconstriction, caused by a cold stimulus, wore off in the course of about five minutes   The hand blood flow of a rested subject, at the beginning of a series of experiments, would therefore not be restricted by cold stimuli arising in remote skin areas, and no removal of such vasoconstriction could occur during the first period of heating   Vasodilatation was, in fact, observed during the first experiment in every series, and its extent did not differ significantly from that observed in later experiments in the same series   Furthermore, as will be seen from Fig 2, heating has been carried out at room temperatures ranging from 20°C to 28°C , and the response at the lowest temperature was no larger than those at higher temperatures

It is considered likely, for these reasons, that the removal of cold stimuli cannot satisfactorily explain the observed phenomena, and that vasodilatation in response to stimulation of the skin by heat (as opposed to the mere withdrawal of cold) has been demonstrated   This conclusion is at variance with the results of a number of other workers (9, 16, 18, 19, 22, 23, 24), and it is necessary to attempt to reconcile these differences   They may be due, in part, to the form of heating which has been employed   A cabinet heated by lamps has been used by some (16, 23), but the skin of the subject was not, in these cases, directly exposed to the radiation   In other studies (5, 9, 18, 19, 22, 23, 24) warm or hot water has been used as a stimulus, being applied to the feet or the forearm and hand   It is possible that the manœuvres involved in immersing the limb, or the unpleasant sensation which results

if the water is too hot, may produce a psychic or reflex vasoconstriction in the control limb (18, 23, 24) If the skin temperature or, to a less extent, the Stewart calorimeter were being used as an index of peripheral blood flow in the control limb, the constriction might not produce a noticeable effect itself, but would delay the recognition of the start of vasodilatation Failure to elicit vasodilatation on heating the occluded limb in water above 42°C (5, 9, 19) may be due to the considerable pain which is caused by this procedure Duthie and Mackay, for example, were forced to terminate the heating " when the pain became intolerable " (5)

It is thought that the application of a comfortably warm stimulus to a relatively large skin area produces nervous reflex vasodilatation in the hand more readily than does strong heating of a small skin area

SUMMARY

1 The time of onset of vasodilatation in the hand, in response to heating the skin of the trunk by means of a radiant heat cradle, has been determined in six subjects, and was found to be between 10 and 15 sec

2 The time of onset of vasodilatation is independent of the rate of heating, within wide limits

3 Heating of the legs alone was found to cause a vasodilatation in the hand of similar latency, and this response was unaltered when cuffs round the thighs were inflated to 200 mm Hg

4 The vasodilatation was associated with simultaneous falls in mouth and rectal temperatures

5 It is concluded that under the conditions existing in the experiments described, vasodilatation in the hand resulted from afferent nervous impulses arising in the heated skin of the trunk or legs

6 The results are discussed in relation to previous observations

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1 2 seconds
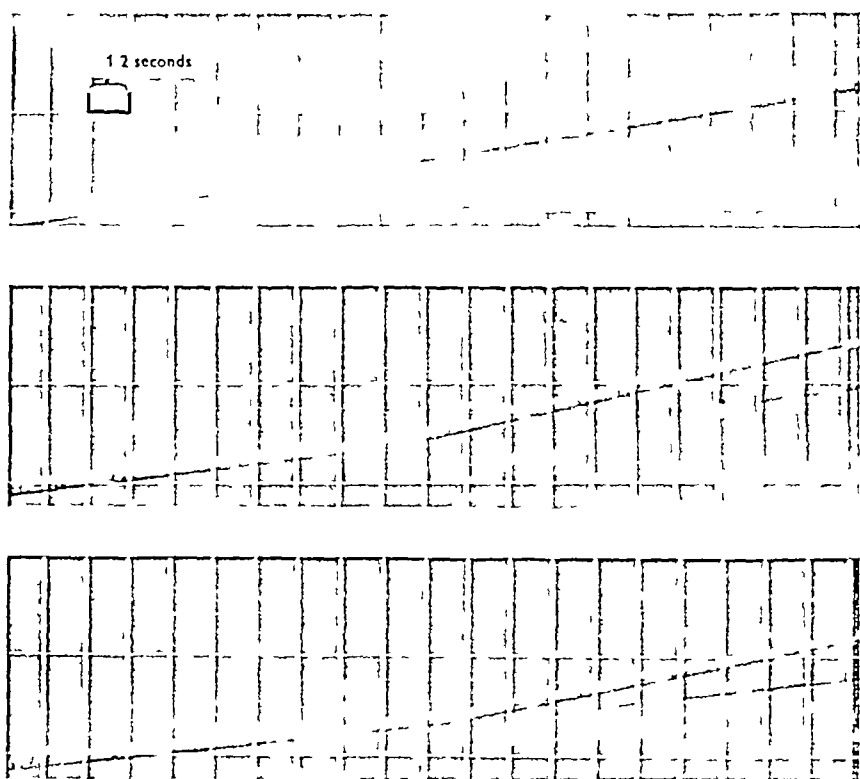
Plate 1   Records of hand volume showing the onset of vasodilatation   Upper record, control
Middle record, low illumination (350 watts)   Lower record, high illumination (700 watts)
Lamps turned on and collecting cuff inflated at start of record   Time marker, 1 2 sec

THE BLOOD FLOW THROUGH THE CALF AFTER EXERCISE IN SUBJECTS WITH ARTERIOSCLEROSIS AND CLAUDICATION

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VARIOUS ergographs have been devised to determine the claudication time in subjects with intermittent claudication, but so far no attempt has been made to study the calf blood flows following the exercise   Observations have, therefore, been made on 24 cases of arteriosclerosis   These were not selected cases, but were the first 24 patients referred to this department for routine examination of their peripheral circulation   Twenty-three were non-diabetic males, and one was a diabetic female   With the exception of this female and one male, each of whom had a pregangrenous condition in one toe, the skin in all cases was healthy   The primary complaint of all these patients was intermittent claudication

Methods

An ergograph has been devised in which the patient, by depressing a footboard on plantar-flexion, raises a 5 5 kg weight   The movement of the footboard is transmitted to an indicator , a mark is placed on a scale behind the indicator and by pushing to this mark through an angle of 33° the subject lifts the weight a distance of 26 cm   By arranging the pointer of a metronome to move in parallel with this indicator the subject, following the time of the metronome, works at a constant rate   This constancy allows accurate comparison with subsequent tests   The footboard is so arranged that the axis of its movement passes through the ankle joint as in walking, and as the leg is kept horizontal with the thigh at an angle of about 60° to the leg, the posterior calf muscles perform the most work   In addition, the incorporation of a free wheel between the footboard and the 5 5 kg load allows work to be performed on plantar-flexion only , the controlled descent of the weight by a suitably adjusted brake drives the footboard back in time with the metronome, so returning the foot to the dorsiflexed position   This has the advantage that it allows alternate contraction and relaxation of the muscles, the posterior calf muscles being

relaxed on dorsiflexion instead of having to resist the descent of the weights This is important because it leads to a wide demarcation between the amount of work that can be done by the normal and the arteriosclerotic The normal adult male of whatever build can make 500 contractions at a rate of 24 per minute and shows no sign of fatigue or pain at the end of this time The arteriosclerotic with mild symptoms of claudication can make about 150 to 200 contractions, while with more advanced disease, the patients are forced to stop with intolerable pain after about 35 to 80 contractions The pain produced on the ergograph was in all cases similar to that produced by walking

In order to record the blood flows through the calf of the leg after exercise a celluloid plethysmograph with air recording was used This has the advantage that it causes the patient no discomfort The calf volume was measured by water displacement An 80 ml recording unit was adopted because of the large post-exercise flows obtained in normal subjects After the plethysmograph had been applied and the patient suitably adjusted on the ergograph, resting calf blood flows were recorded until a steady base line had been obtained Exercise was then begun at a rate of 24 contractions a minute and continued until pain forced the patient to stop The number of contractions carried out is the claudication index The first flow was recorded on cessation of exercise, and it was possible in all subjects for this recording to begin within a second of exercise ceasing Thereafter, records were obtained at 10, 20, 30, 45, 60 sec , then at $\frac{1}{2}$ to 2 min intervals There was one change in technique from that usually adopted in recording calf flows It is customary to inflate an ankle cuff to just above systolic pressure to cut out the foot circulation about 30 sec to 1 min before recording a calf blood flow As the end point of the exercise cannot be determined beforehand, and as this procedure causes an abnormal shortening of the claudication time, the ankle cuff was inflated to 80–90 mm Hg simultaneously with the collecting cuff (3)

Results

Grant (2) has pointed out that any material departure from the resting forearm blood flows resulting from local exercise can be safely attributed to a change in muscle circulation It is probable that the same is true of the calf and that post-exercise recordings represent mainly muscle blood flow

The behaviour of the calf circulation in a normal individual following this type of exercise is illustrated in Fig 1 This and the other normal individuals were from the same age group as the patients It was found that 500 contractions could be carried out without symptoms Normal pulsations were present in the plethysmographic record The immediate post-exercise flow was high, and this was followed by a very rapid decline to the resting level In addition, when ischaemic exercise was carried out

with an 8″ thigh cuff inflated to 200 mm Hg and then released just before the first recording, the immediate volume flow of blood was well above that obtained after 500 normal contractions, indicating that a considerable potential reserve was available
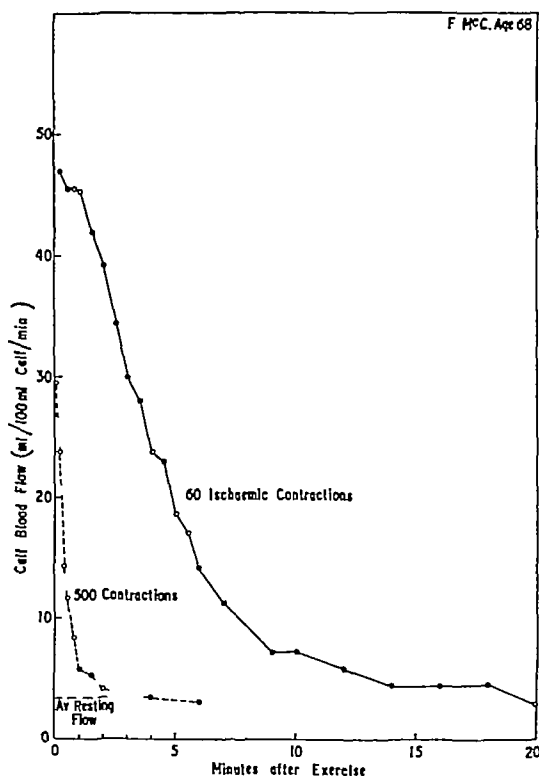
Fig 1 Normal pattern for calf circulation Normal pulsations on plethysmogram 500 contractions left calf without symptoms Initial high flow with a rapid recovery to the resting level With 60 ischaemic contractions left calf the immediate post-exercise flow is higher than with 500 normal contractions, indicating the potential reserve available

The results obtained in the 24 cases studied can be divided into two main groups, A and B, in both of which the number of contractions which could be carried out was well below 500 There was no age differentiation between the two groups

Group A These subjects showed pulsations in the plethysmographic record even though these were usually less than normal The immediate post-exercise flow, though lower than in normal individuals, was higher than any which followed it, and there was a gradual slow decline to the resting level Further, ischaemic exercise carried on to claudication did not increase the immediate post-exercise flow (Fig 2) Twelve out of the 24 cases fell into this group
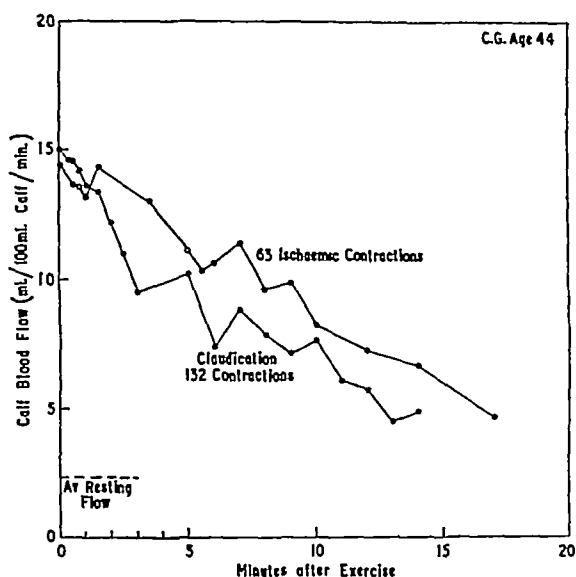
Fig 2 Example of a Group A pattern Slight pulsations on plethysmogram Only 132 contractions of left calf possible owing to pain The immediate post-exercise flow is less than normal and the height of this flow is not increased by ischaemic exercise Notice the very slow return to the resting level The immediate post-exercise flow is, however, as in the normal, higher than the flows which follow it
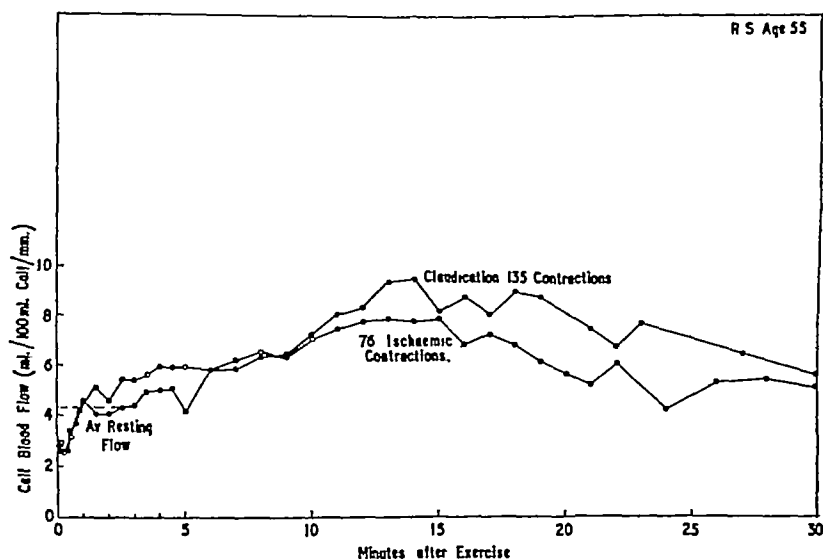
Fig 3 Example of a Group B pattern Absent pulsations on plethysmogram Only 135 contractions were possible owing to pain Note the low flow immediately after exercise with the subsequent increase, the maximum flow only being reached about 14 mm after cessation of the exercise The same phenomenon is present after 76 ischaemic contractions of the same muscles, and the initial flow is the same in both cases Note also that the immediate post-exercise flows are lower than the average resting flow

Group B   These subjects showed complete or almost complete absence of pulsations in the plethysmographic record   Immediate post-exercise flows were much lower than normal, but instead of being higher than any that followed, the flows gradually increased to reach a maximum some time after cessation of exercise   This pattern and the extent of the initial flow were unchanged if the exercise was carried out with the circulation occluded in the thigh   (Figs 3 and 4)   Twelve out of the 24 cases fell in this group Table I gives details of the results obtained on them, and the remainder of this paper deals only with this group
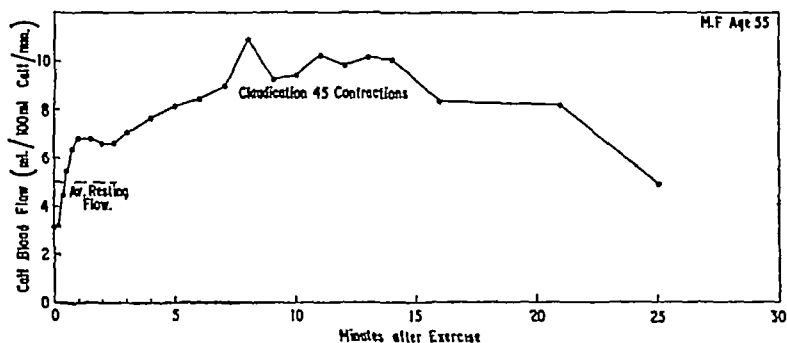
Fig 4   Example of a Group B pattern, with absent pulsations on the plethysmogram   Note the gradually increasing flow after exercise and that the initial flow is depressed below the resting blood flow level

TABLE I

Patient	Average resting flow	Immediate post-exercise flow	Maximum post-exercise flow	Time taken to reach maximum (minutes)
R S	4 3	2 6	9 4	14
E G	4 9	4 0	10 8	9½
J.McL	7 7	7 5	10 8	9
M.F	5 0	3 1	10 8	8
W.L	3 8	9 0	19 4	7½
W.McG	3 3	6 7	12 3	7
J.McC	1 8	6 5	15 8	4
S C	5 4	5 3	9 0	3½
W S	3 8	9 5	17 5	3
F.F	2 8	12 0	18 6	2½
W Y	1 3	4 5	10 0	1½
A J	1 7	12 9	16 8	1

Blood flows are expressed in ml./100 ml calf/min.

In 3 of these subjects the calf blood flow immediately after exercise was below their average resting level   Fig 5 shows the plethysmographic tracing from one of these cases   In 7 subjects, the flow immediately after
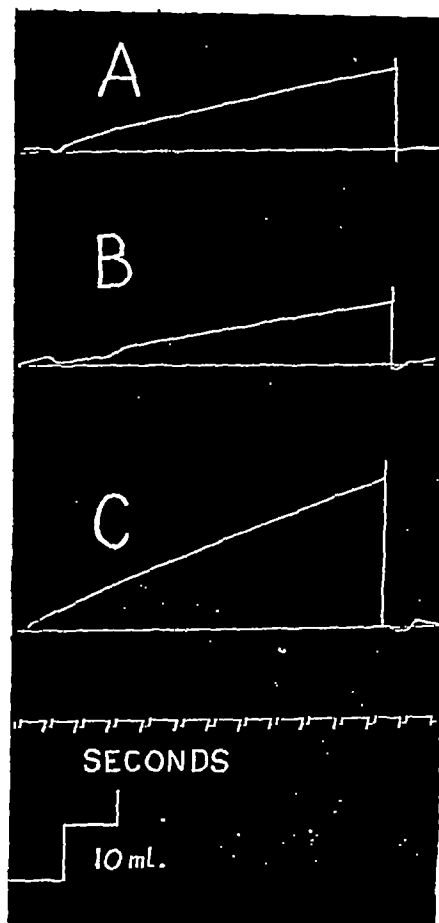
Fig 5   Plethysmographic records of blood flow from a subject in Group B
A   Resting calf blood flow (5 5 ml per 100 ml calf per min)
B   Calf blood flow immediately after exercise (4 1 ml per 100 ml calf per min)
C   Maximum calf blood flow, 8 min after the end of exercise   (10 4 ml per 100 ml calf per min)

exercise was higher than the average resting flow and in 2 it was about the same   When the former 3 subjects were again exercised to claudication immediately their initial flows after exercise had reached a maximum, the immediate flow following the second exercise again fell below the resting level (Fig 6)   The same experiment carried out in 4 of the 7 subjects mentioned above, while it did not depress their flows below the resting level

after the second exercise did depress them well below the maximum post-exercise level   For example, on one of these latter cases the resting flow was 4 1 ml /100 ml calf/min , exercise was carried out to claudication, the first post-exercise calf flow was 8 ml and 90 sec later the flow had reached 15 9 ml   At this point exercise was re-commenced and again continued to claudication   The flow immediately following this was 6 7 ml reaching 15 4 ml 105 sec later
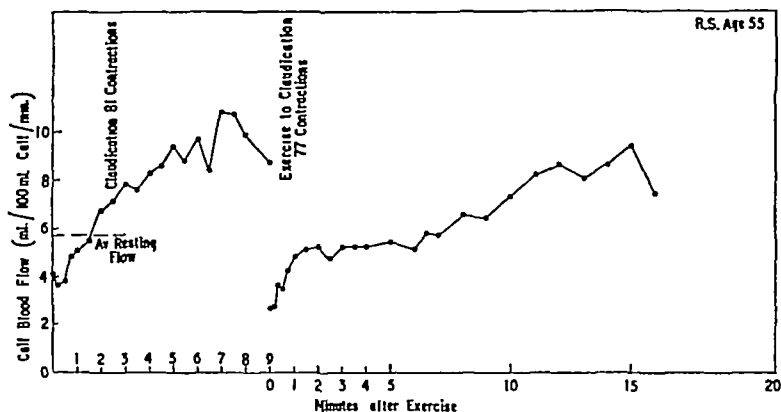
Fig 6   Group B, absent pulsations in plethysmogram   This demonstrates that when post-exercise flows have reached a maximum a further period of exercise again causes a decrease in calf blood flow   The first series was recorded after exercise to claudication (81 contractions)   Exercise was begun again carried to claudication after 77 contractions and the second series of blood flows measured

This finding of the gradual increase in flow after cessation of exercise was a constant one   One of these patients was tested ten times over a period of 16 weeks, another weekly for 10 weeks and they never failed to show it   In only one case was it absent on a second testing   This was a patient who had previously shown a well marked gradual increase in flow following exercise carried on to claudication, and had no evidence of this after consuming 6 glasses of whiskey and 6 bottles of stout prior to a second test

In 8 of these 12 cases the circulation in the opposite calf was tested Two showed a Group B response, 3 showed a Group A response and in 3 the calf circulation appeared to be normal   In 2 of these latter cases a comparison was made of the blood debt in the good and bad calf by carrying out a similar number of contractions with both legs, the circulation being arrested in each by a 7″ thigh cuff inflated to 200 mm Hg   On release of this cuff after exercise the calf blood flows were recorded until the resting level was restored In the first of these patients the good calf had 72% of the debt of the bad one, and in the second the bad calf had 75% of the debt of the good one There does not seem, therefore, to be any great difference in the blood debt

The exercise carried out by the patients in this group usually caused a transient rise in general blood pressure   A fall in this pressure was never observed   The presence or absence of the ankle cuff made no difference to the shape of the blood flow curve following the exercise   Some of these patients, after the time taken for the pain to come on had been determined, were again exercised to just before this point, but this did not alter the type of result obtained   Further, a period of 6–10 min ischaemic without exercise induced by a thigh cuff at 200 mm Hg at the end of which time no pain was present in the leg, produced a typical Group B pattern (Fig 7) The severe pain produced by continuing the exercise as long as possible did not seem to cause any voluntary or involuntary tightening of the muscles No difference in the muscle relaxation could be detected by palpation of the calf before and after claudication
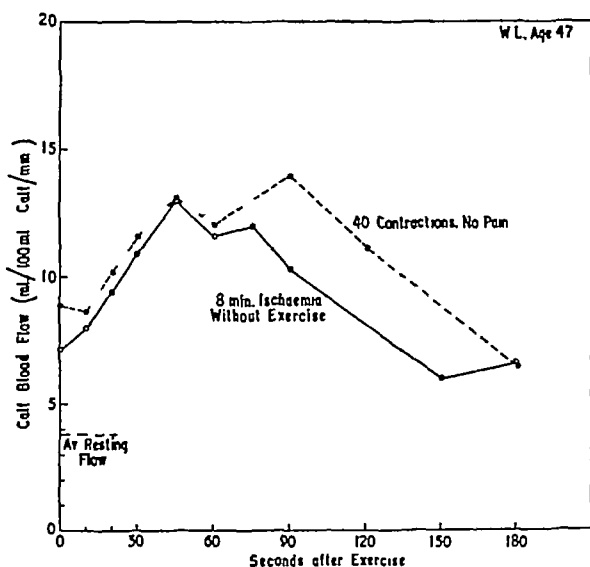
Fig 7   This shows the calf blood flows increasing after exercise in the absence of pain, and that a period of ischaemia without exercise can produce a similar type of response

Of these 12 cases, 3 had had a lumbar sympathectomy performed 3 weeks to 4 months previously   In one the skin temperature of the toes and skin resistance of the leg showed no change in response to indirect heating carried out by immersing the hand, forearm and as much of the arm as possible in a stirred water bath at 44 5°C and there was, therefore, no evidence that the sympathectomy was incomplete   One case, tested before sympathectomy, still showed the phenomenon after operation   In 5 of the remainder neither indirect heating, nor the injection of 400–500 mg tetraethylammonium bromide intravenously just before cessation of exercise, nor paravertebral block had any effect on the pattern of the blood flow following the exercise

DISCUSSION

While the mechanism responsible for the gradual increase in calf blood flow after cessation of exercise has not been discovered, certain possibilities can be excluded. Changes in general blood pressure can be ruled out as this generally rose and never fell following the exercise. Pain is not a factor as the same results are obtained in its absence. In addition, the patients in Group A did not show this low post-exercise flow, even though the pain produced by exercise was probably as severe as that experienced by the patients in Group B. Reflex vasoconstriction due to inflation of the ankle cuff is not the cause as it still occurs in the absence of this cuff. The possibility of reflex or voluntary muscular contraction due to the pain and passing off with it was considered. If the muscles were contracted in this way they might either reduce the arterial inflow (1) or, by compressing the veins, prevent regular venous filling during the recording of the flows. As the phenomenon is independent of pain, however, it must be independent of the consequence of pain. To palpation, the muscles were as relaxed following the exercise as they were before it commenced.

The sympathetic nervous system does not appear to be responsible for these findings, as the pattern of the blood flows after exercise was unchanged following sympathectomy and other methods designed to release sympathetic control of blood vessels.

The immediate flow following the exercise might represent the flow through the main artery and the gradual increase which follows might be due to the opening up of collateral anastomoses. If this were the explanation, then if exercise was recommenced after the initial post-exercise flows had reached a maximum, the immediate flow following the second bout of exercise should be at least as high as this previous maximum. This did not happen, the calf flow following the second exercise was again markedly depressed. (Fig 6)

There are 3 remaining possible explanations for these findings —

(1) A drop in perfusion pressure in the limb, due to obstruction in the main arterial supply and the muscle vasodilatation consequent upon the exercise. For example, if an obstruction was present high up in the main artery going to the limb, exercise of such a limb would lead to a dilatation of the muscle blood vessels and a consequent lowering of the pressure distal to the obstruction. In the event of a second obstruction being present in the artery just proximal to the blood vessels leading to the calf muscles, blood could be diverted into muscle vessels proximal to the calf which are inevitably exercised to some degree during these experiments. If a marked local fall in arterial pressure did occur in the limb following the exercise it could be argued that the use of too high a collecting pressure might simulate these results by reducing the arterial inflow. The same decrease in flow can still be obtained, however, with a collecting pressure as low as 20 mm Hg.

(2) Vasoconstriction followed by vasodilatation of the calf vessels

(3) A combination of diversion and vasoconstriction

While various experiments have been carried out in an attempt to decide between these possibilities no definite conclusions have as yet been reached

Finally the immediate flow after exercise may be supposed to give an indication of the arterial inflow to the exercising muscles during phases of relaxation and there is no evidence which suggests that the inflow during contraction is greater than during relaxation   It therefore appears likely that in these cases in Group B there was a reduced calf blood flow during the exercise as well as immediately afterwards

SUMMARY

1   An ergograph has been devised which, used in conjunction with a celluloid plethysmograph, enables the exercise tolerance of the posterior calf muscles to be assessed and the post-exercise calf blood flows to be determined

2   Using this technique, 24 unselected cases of arteriosclerosis with claudication have been studied   In 12 of these patients it was found that the calf blood flows failed to reach their maximum after exercise until some time had elapsed, this time varying from 1–14 min with an average of about 6 min

3   This phenomenon could be due either to diversion of blood into muscles proximal to the calf, constriction followed by dilatation of the calf vessels, or a combination of these two mechanisms   The evidence at present available does not allow a conclusion to be reached about the mechanism concerned

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THE CIRCULATORY CHANGES ASSOCIATED WITH ANEURYSM OF THE AXILLARY ARTERY AND CLUBBING OF THE FINGERS

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UNILATERAL clubbing of the fingers is uncommon, but interesting because of the information that may be gained concerning the circulatory changes associated with finger clubbing, as the normal hand is available for comparison   Recent reviews (10, 11) show that unilateral clubbing is most frequently associated with an aneurysm of the main artery of the limb, either at its origin or in the proximal part of its course   The present report is concerned with gross unilateral clubbing of the fingers and aneurysm of the distal part of the axillary artery

Case report

A male aged 63 years, married with no children, served in the Royal Marines from 1903 to 1919 and subsequently was employed as a general labourer   In 1912 he developed a penile sore for which he received treatment with mercury over a period of several months   Thereafter he remained well until 1938 when a painless swelling appeared on the forehead   This was incised on several occasions, failed to heal satisfactorily, and was later diagnosed as a gummatous ulcer   The condition subsequently responded to antisyphilitic injections and healed with much scarring   In 1947 a lump was noticed one evening in the right armpit   There was no pain but the patient attributed the condition to straining himself a few hours previously by reaching up to lift a heavy box from a lorry   No weakness or any other disturbance was noted in the limb   The swelling has persisted unchanged to the present date   In January, 1949, he noticed increasing abdominal distension and later swelling of the genitalia and legs   Abdominal paracentesis has subsequently been carried out repeatedly at intervals of two to six weeks   His general condition has recently considerably deteriorated, his appetite has been poor and he has lost weight in spite of fluid retention   He has had a cough and sputum for many years especially during the winter months, and has become increasingly short of breath on exertion. There was formerly a heavy spirits drinker (up to a bottle a day) but has been moderate in recent years

On examination there was an irregular depressed white scar over the right side of the forehead.   A few telangiectases were present on the face and purpuric spots were present on both arms   There was no abnormal lymph node enlargement in the neck, axillæ or groins   The fingers of the right hand were conspicuously clubbed with definite curving of the nails, fluctuation of the nail bed and bulbous swelling of the pulp of the finger tips   The fingers of the left hand showed changes suggestive of very early clubbing—slight fluctuation of the nail bed and curving of the nails (Fig 1)   After exposure for half an hour to ward temperature the right hand was always warmer than the left   The veins of the dorsum of the right hand also appeared slightly more prominent

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A firm pulsating swelling measuring approximately 5 × 4 cm in diameter was present in the right axilla, and apparently arose from the third part of the axillary artery (Fig 2) The pulsation was abolished by compressing the subclavian artery, but the swelling was not thereby reduced in size The brachial and radial pulses on the two sides were of equal force No cardiac abnormality was detected The chest was emphysematous with poor movement, faint breath sounds, generalized rhonchi and moist sounds at both bases The abdomen was grossly distended with fluid The upper limit of liver dullness was in the 6th space the liver edge was not palpable even after drainage of the fluid Rectal examination was normal Considerable œdema of the sacrum, genitalia and ankles was present No abnormality was detected in the nervous system

Radiological examination of the chest showed moderate bilateral basal emphysema Diffuse fibrosis was present in both upper zones, the upper part of the right lower zone and the left mid zone The heart was not enlarged but a moderate prominence of the aortic knuckle was noted X ray of the right arm showed a soft tissue opacity in the axilla associated with minimal calcification X ray of the hands showed enlargement of the subungual cancellous tufts of certain of the terminal phalanges These changes were much more conspicuous on the right side No subperiosteal new bone formation was seen along the shafts of the metacarpals or distal forearm bones

Urine Albumen, nil, sugar, nil *Blood* Wasserman and Kahn reactions strongly positive Thymol turbidity, 4 units Serum bilirubin, 0 2 mg /100 ml Blood Hb, 13 4 g /100 ml R B C 4,400,000/cu m W B C 6,500/cu m , polymorphs 70%, eosinophils 2%, lympho cytes 20%, monocytes 8% Plasma protein, 6 5 g /100 ml , albumen 2 5 g , globulin 3 8 g /100 ml Plasma prothrombin 67% of normal Serum cholesterol 138 mg /100 ml

The clinical diagnosis was cirrhosis of the liver, syphilitic aneurysm of the axillary artery and pulmonary fibrosis, possibly syphilitic in origin

The opportunity was taken of making further detailed observations on the circulation in both upper limbs

Methods

Brachial artery blood pressures were estimated on the two sides by the auscultatory method using similar sphygmomanometers The readings were made as far as possible simultaneously and half way through the determinations the observers changed sides

Digital artery systolic blood pressures were estimated by fitting cuffs 2 cm in width around the proximal phalanges and plethysmographs over the distal two phalanges By means of an entirely air conducting system through wide bore rubber tubing the pulsations of the finger tip were transmitted to a soap bubble volume recorder (4) and photographed By inflating the cuffs above systolic pressure and reducing the pressure slowly it was possible to determine the level at which pulsations first appeared in the finger tip This was taken to represent the systolic pressure in the digital arteries at the base of the finger As it was not possible to photograph records from two soap bubble volume recorders simultaneously the following procedure was adopted Both plethysmographs were connected by tubing of equal length and bore through a Y piece to one bubble recorder and clamps were fitted to shut off each limb of the Y in turn To change the reading from one finger to the other required about 10 sec The pressure in the finger cuffs was reduced until pulsations were seen and recorded from one finger Switching over to the opposite side it was then possible to demonstrate complete absence of pulsations and on returning to the first side to show that pulsations were still present

The venous pressures were measured relative to the sternal angle in two corresponding forearm veins with citrate manometers after the patient had been lying at rest for half an hour with the arms exposed Between the readings the arms were placed in different symmetrical positions to reveal any changes due to local compression

Venous occlusion plethysmographs were used for recording the blood flow through the forearms and hands All the readings were made with water in the plethysmographs maintained at 34°C The forearm blood flows were recorded with the hand circulation excluded by wrist cuffs inflated above arterial pressure (200 mm Hg) The tracings from the two sides were recorded simultaneously and every precaution was taken as regards position of the limb, placing of collecting cuffs, and amount of tissue included in the plethysmograph to ensure that results from the two sides were comparable Matched volume recorders writing on a smoked drum were used for tracing the inflow curves After a series of observations the volume recorders were switched over to opposite sides and at the conclusion they were separately calibrated

Heat elimination from the two hands was determined simultaneously in two Stewart's calorimeters as modified by Greenfield and Scarborough (3) The two thermometers were calibrated against each other and the difference between them over the range of 27–31°C was less than 3% Before observations were begun both hands were soaked for 25 minutes in a basin of stirred water from which the calorimeters were subsequently filled This procedure was designed to exclude temperature differences on the two sides due to external causes

Venous blood samples for gas analyses were taken simultaneously under paraffin from two corresponding antecubital veins The limbs were exposed and at rest for one hour before the specimens were taken and congestion of the limbs avoided before and during withdrawal of the blood The subsequent gas analyses were carried out in the manometric Van Slyke apparatus

Results

The brachial artery blood pressures with the patient at rest in bed, as determined from the averages of three sets of two independent observers, were, right 133/85, and left, 130/80

The pressure in the digital arteries of the middle fingers was determined by repeated observations on three different occasions with the patient sitting in a chair The pressure on the right side was always found to be higher than that on the left This finding was constant at different degrees of peripheral vasodilatation obtained by heating the patient The average of these results showed the systolic digital pressures to be, right 145 mm , and left 128 mm

The venous pressures on the two sides showed no consistent differences and the difference between the two readings was never greater than 2 cm of citrate solution   The averages of the readings in different positions were, right + 1 7 cm , and left + 0 7 cm

Forearm blood flows were measured on two separate occasions   The flow through the right forearm was slightly greater than through the left The results were

TABLE I
Forearm blood flow

Date	No of readings	Right		Left	
		Forearm vol ml	Blood flow ml /100 ml /min	Forearm vol ml	Blood flow ml /100 ml /min
16 6 49	20	525	1 8	515	1 7
19 8 49	23	555	2 4	560	2 2

Of the 43 pairs of readings, 1 pair was identical, and in all the remaining 42 the flow was greater on the right side

Hand blood flows as determined by plethysmographs showed that in every tracing the blood flow through the right hand was considerably greater than that through the left (Fig 3)   The hands were inserted into the plethysmographs to corresponding anatomical levels, but the right with the clubbed fingers was slightly larger
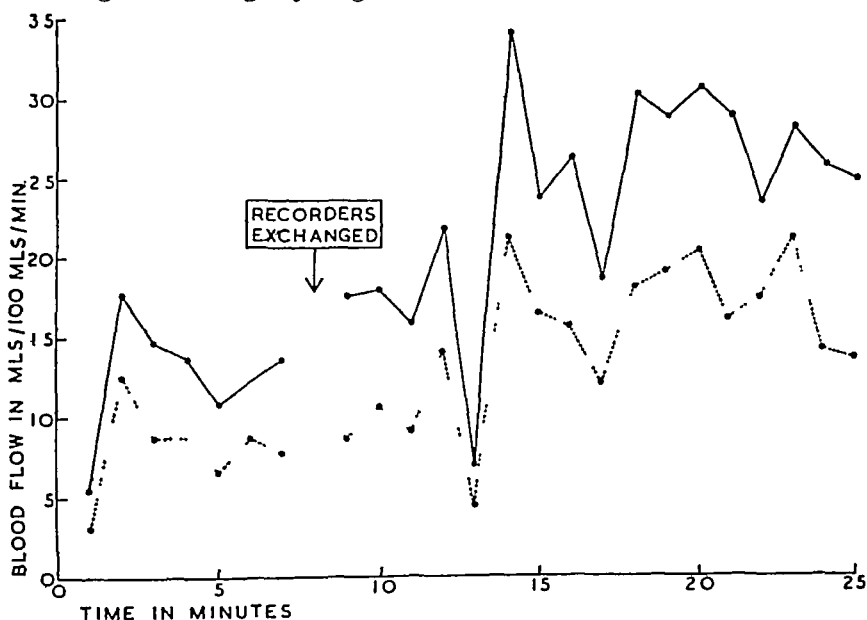
Fig 3   Graph of simultaneous hand blood flows by venous occlusion plethysmography Continuous line right hand, dotted line left hand

The results were

TABLE II
Hand blood flow

No of readings	Right		Left	
	Hand vol ml	Blood flow ml /100 ml /min	Hand vol ml	Blood flow ml /100 ml /min
25	400	21 0	385	12 8

The heat elimination was consistently greater from the right hand throughout the period of observation, both with the subject sitting at rest and with the feet in hot water to produce vasodilatation in the hands (Fig 4) The results may be summarized
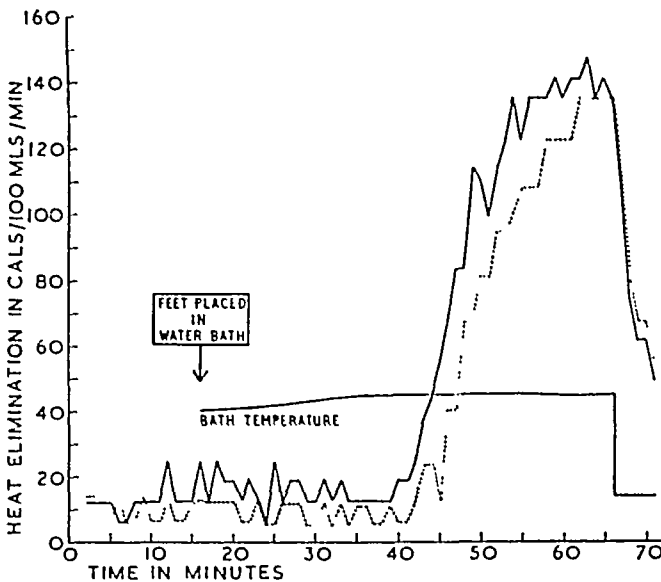
Fig 4 Simultaneous heat eliminations from both hands Continuous line right hand dotted line left hand. Thermometer readings at 1 mm intervals The ordinate for the footbath temperature in degrees centigrade uses the same numbers as the heat elimination scale

TABLE III
Hand heat elimination

Duration of observations min	Right		Left	
	Hand vol ml	Heat elimination cal /100 ml /min.	Hand vol ml	Heat elimination cal /100 ml /min
70	320	52 3	290	44 0

The analysis of the blood samples withdrawn from the antecubital veins was carried out in duplicate by Dr J Hardwicke with the following results

	Right	Left
Oxygen vol per cent	8 4	7 2
Carbon dioxide vol per cent	40 8	45 5

No abnormality was demonstrable in the sympathetic nerves of the upper limbs   The vasomotor reflexes on the two sides were similar and entirely normal responses to placing the feet in hot and cold water were obtained   Sudomotor and pilomotor activities were also unaffected as was shown by normal sweating on the affected side and by the development of the "goose-skin" reaction on pinching the trapezius

Discussion

The slight degree of clubbing in the left hand is probably to be attributed to the cirrhosis of the liver and to the pulmonary fibrosis, while the greatly increased clubbing in the right hand is largely a consequence of the axillary aneurysm   Several cases of unilateral clubbing of the fingers have been described in association with an aneurysm of either the subclavian or axillary artery (6, 10)   The majority of these aneurysms have been in the region of the thoracic outlet and have been complicated by features arising from pressure on neighbouring nerves and veins   Syphilitic aneurysms of the distal part of the axillary artery are extremely rare   The case described is thus of exceptional interest, particularly as the swelling was easily accessible for clinical examination, and disturbance of neighbouring structures that might play a part in the production of the marked unilateral clubbing can be excluded   In some of the older case reports the hand distal to an aneurysm has been described as cold, blue, and swollen (8)   These cases, as far as can be ascertained from the published records, have always been complicated by disuse of the limb resulting from brachial plexus lesions, by pressure on the subclavian vein, or by apparent complete obstruction of the main arterial supply to the limb   In such cases the mechanism of the production of the clubbing is obscure and it has been variously attributed to disturbance of the sympathetic innervation or to rise in venous pressure   Furthermore, in many of the older reports there is no clear distinction drawn between clubbing, hypertrophy and œdema

The results of the present investigation have all consistently shown that the circulation through the right hand was considerably increased in comparison with the opposite side   This increase in blood flow on the right side was considerably greater than the variation observed in the comparison of the two sides in the normal subjects by Cooper and others (2)   The instrumental readings have thus confirmed the clinical observation that the conspicuously clubbed hand was always the warmer

It should be noted that the greater blood flow was associated with an increased oxygen tension in the venous blood on the right side. It was not possible to obtain the blood samples at the same time as the limb blood flow was being estimated and therefore results cannot be correlated with certainty. If, however, it is assumed that the arterial oxygen saturation on the two sides is equal, that the blood flow determinations quoted above afford a representative picture of the proportionate increase on the right side, and that blood taken from corresponding antecubital veins gives a reasonable mixed venous sample of forearm and hand blood, then it can be concluded that the blood flow on the right side is considerably increased above the metabolic requirements of the part

Brachial artery blood pressure measurements on the two sides with the sphygmomanometer showed no significant differences. It was, however, realized that this method was not ideal, for when the cuff is inflated the flow through the artery is stopped, and if it is to be argued that the presence of an aneurysm in some way alters the brachial artery flow it would be highly desirable to obtain pressure measurements with the arterial flow unobstructed. Digital artery pressures by a cuff method were consistently higher on the right side. These results suggest that there was considerable peripheral arterial vasodilatation on the more conspicuously clubbed side

Similar clinical observations regarding the increased temperature of a unilaterally clubbed hand developed in association with a pulsating syphilitic arterial aneurysm and unobstructed distal circulation have been made in the past by several authors (7, 10). Richards and Learmonth (9) recorded a similar finding of increased warmth in a foot distal to a syphilitic popliteal aneurysm, and confirmed their clinical observations by skin temperature readings. These authors made no mention of the condition of the toes of their patient but Sartor (11) has reported a case of clubbing of the toes secondary to syphilitic aneurysm of the femoral artery. Brooks (1) has described a case of unilateral finger clubbing in association with an aneurysm of the proximal part of the axillary artery. Estimations of the venous oxygen saturation were made in the two limbs and the venous blood on the clubbed side was found to have a higher oxygen content. In this respect his result was similar to that in our case. No other measurements of the circulation in a limb with an arterial aneurysm and clubbing have apparently been reported. Mendelowitz (7) investigated the circulatory changes associated with bilateral clubbing of the fingers but his series did not include calorimetric observations on any case of unilateral clubbing secondary to a limb aneurysm. In comparison with the hands of normal subjects he described in bilaterally clubbed fingers an increased blood flow and a decreased pressure gradient from brachial to digital artery. His findings in bilateral clubbing arising from the commoner general causes were thus similar to those in our case when the markedly clubbed hand was compared with its relatively unaffected fellow

The evidence obtained from the investigations on the case reported above and from past records shows clearly that the change associated with a pulsating syphilitic aneurysm and unobstructed distal arteries is an increase in the distal circulation   The temporal relationship between the development of the aneurysm, the increased peripheral blood flow and the clubbing of the fingers is of considerable interest   It is generally agreed that the aneurysm precedes and brings about the finger clubbing   The relationship between the increased peripheral circulation and the clubbing is more open to question as no measurements of the circulation have ever been made immediately after the formation of such an aneurysm   It is, however, known that an event which reduces the peripheral arterial flow, such as spontaneous clotting and subsidence of the aneurysm, leads to the disappearance of the clubbing (5, 6)   Surgical excision leads to a similar result (12)

In our case the only abnormality responsible for the development of the marked clubbing on the right side was the presence of the axillary aneurysm   This was associated with an increase in the blood flow beyond it   There was no evidence that the aneurysm had caused any venous obstruction

The physical mechanism whereby such an aneurysm produces an increased distal circulation is difficult to elucidate   A crude model of the arterial circulation was constructed in which it was possible to show that the flow on the side containing a distensible sac was increased only if a valvular mechanism was present at the proximal entry to the sac, so that the added fluid from the elastic recoil of the sac was directed distally   With such a valvular action at the entry of the artery possibly due to increased angulation during diastole the pulsating aneurysmal sac would greatly augment the distal blood flow   In this connexion it is interesting to note that popliteal aneurysms of arteriosclerotic origin are not associated with an increased distal circulation (13)   These aneurysms are usually elongated fusiform dilatations while those of syphilitic origin tend to be more spherical and saccular and thus more likely to produce a valvular angulation at the point of entry of the artery   In the past, attention has been chiefly directed towards the more spectacular and dangerous complications of syphilitic aneurysms such as rupture, distal embolism, and pressure effects   It has not been fully realized that uncomplicated they may be associated with a distal hyperæmia   It is tentatively suggested that this physical augmentation of distal arterial flow, resulting in the fingers receiving more blood than is required for either heat elimination or nutrition of the tissues, leads to the development of the anatomical changes associated with clubbing of the fingers

SUMMARY

A case of cirrhosis of the liver and syphilitic axillary aneurysm associated with increased ipsilateral clubbing of the fingers is described

The circulatory changes distal to the aneurysm consisted of an increased blood flow through the hand and a decreased pressure gradient in the arterial tree as compared with the unaffected side   Thus increased blood flow was associated with a decreased oxygen consumption per unit volume of blood   The marked clubbing of the fingers of the right hand appeared to be associated only with these arterial circulatory changes

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(a)   (b)
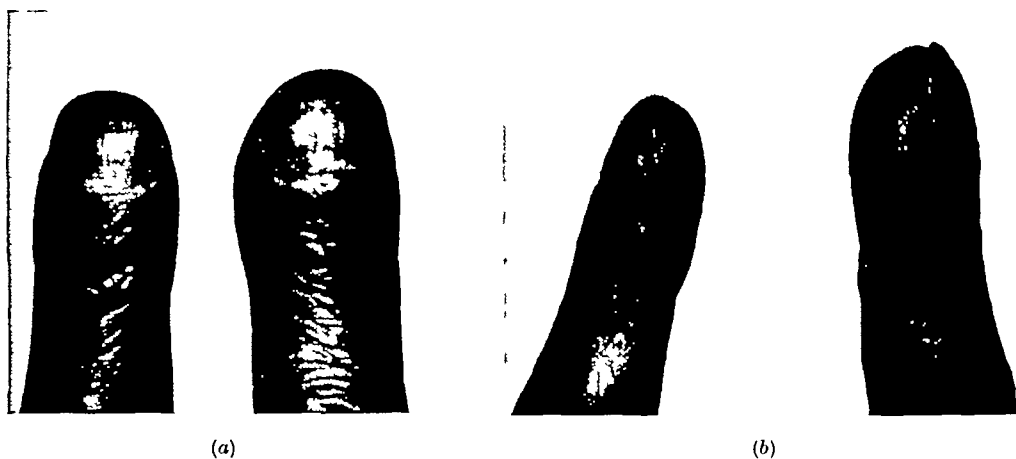
Fig 1   Dorsal and lateral views of right and left index fingers

Fig 2   Axillary aneurysm

THE EFFECTS OF INTRAVENOUS INFUSION OF MIXTURES OF L-ADRENALINE AND L-NORADRENALINE ON THE HUMAN SUBJECT

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Introduction

THE effects of intravenous infusion of adrenaline and noradrenaline in man have been shown to differ in a number of respects   Goldenberg *et al* (8) made a study of pulse rate, pulmonary and systemic arterial pressure, cardiac output and oxygen consumption in normal and hypertensive patients   Adrenaline raised the pulse rate, the systemic systolic and main pulmonary arterial pressures, and the cardiac output   It lowered the total peripheral resistance   Noradrenaline slowed the pulse, raised the systemic and diastolic and pulmonary arterial pressures, lowered the cardiac output, and raised the total peripheral resistance   Barcroft and Konzett (4) confirmed the pulse rate and blood pressure findings, and Swan (12) has shown that while both adrenaline and noradrenaline constrict the hand vessels, adrenaline increases, but noradrenaline reduces blood flow through the muscles

It now seems clear that noradrenaline and adrenaline are both present in the adrenal gland, and may both be released from it   Schumann (11) has produced evidence for the presence of noradrenaline in pigs' adrenal glands   Goldenberg and others (7) have obtained evidence by paper chromatography that extracts of the adrenal medulla of cattle contain l-noradrenaline as well as l-adrenaline, and noradrenaline from this source has been isolated by Tullar (13)   Holton (9) has shown that both noradrenaline and adrenaline are present in human adrenal tumours   Observing the response of the denervated and innervated nictitating membranes and the blood pressure, Bulbring and Burn (5) showed that in the spinal eviscerated cat, stimulation of one splanchnic nerve produced effects which could be matched by mixtures of adrenaline and noradrenaline, but not by either substance alone   They concluded that noradrenaline as well as adrenaline is released from the adrenal gland   The proportion of adrenaline varied considerably from cat to cat and fell with repeated stimulation   Percentages from 98% to 0% were found, most percentages being between 80% and 20%

On the supposition that the human adrenal gland might also discharge a variable mixture of the two substances into the blood stream, it seemed important to investigate the effects of a number of such mixtures infused intravenously   As far as we are aware, the only experiments in which the effects of mixtures have been observed in the human are those of Goldenberg and others (8) who infused mixtures of equal parts

This paper presents the results of infusing a number of mixtures intravenously into normal human subjects

Methods

Observations were made on 5 normal males aged 19 to 32 *   All except one of these had previously acted as subjects for experiments on many occasions   The subject sat in a chair for not less than 50 min before observations started   During this time the plethysmograph and various pneumatic cuffs were applied, and during the last 10 minutes the intravenous ascorbic acid and saline infusion was started   The room temperature was maintained at $24 \pm 1°C$, and the subject wore normal indoor clothing

The rate of inflow of blood to the right calf was measured with a plethysmograph filled with water at 33-34°C, and provided with a through-and-through cuff   The occluding cuff distal to the plethysmograph was inflated to 200 mm Hg 5 25 min before each drug infusion, and maintained at this pressure until 3 minutes after the infusion was completed, a total of 13 25 min (10)   The rate of blood inflow to the right hand was measured with a plethysmograph filled with water at 31-32°C, the hand being inserted into a loosely-fitting surgical glove sealed to the plethysmograph opening   The hand was supported entirely above a horizontal line through the sternal angle   The collecting cuffs below the knee and above the wrist were inflated from a common reservoir at 70 mm Hg   Blood flows were recorded at $\frac{1}{2}$ minute intervals during the observation period , the collecting cuffs were inflated for 13 seconds on each occasion

Arterial pressure was measured every minute in the left arm by auscultation   The pneumatic bag was inflated to about 200 mm Hg from the reservoir immediately after a blood flow observation, so that any effects of such an inflation on the next observation should be as small and as constant as possible   The pulse rate was determined twice a minute by counting the number of pulse waves in 10 sec on the plethysmograph tracings

Solutions were prepared usually within 30 min and always within 60 min of their infusion   0 9% sodium chloride containing 0 001% ascorbic acid was used for the continuous intravenous infusion, and for making up the drug dilutions   Gaddum, Peart and Vogt (6) state that in the presence of this concentration of ascorbic acid, dilute adrenaline and noradrenaline solutions are stable for many hours   Adrenaline solutions were prepared

Results

The results of one of the 12 experiments are shown in Fig 1   The effects of the infusions on the calf blood flow, the systolic and diastolic blood pressure and the pulse rate are clearly shown   In the case of the hand blood flow, however, the base line fluctuations are so large that it is difficult to assess the degree of change due to the infusion
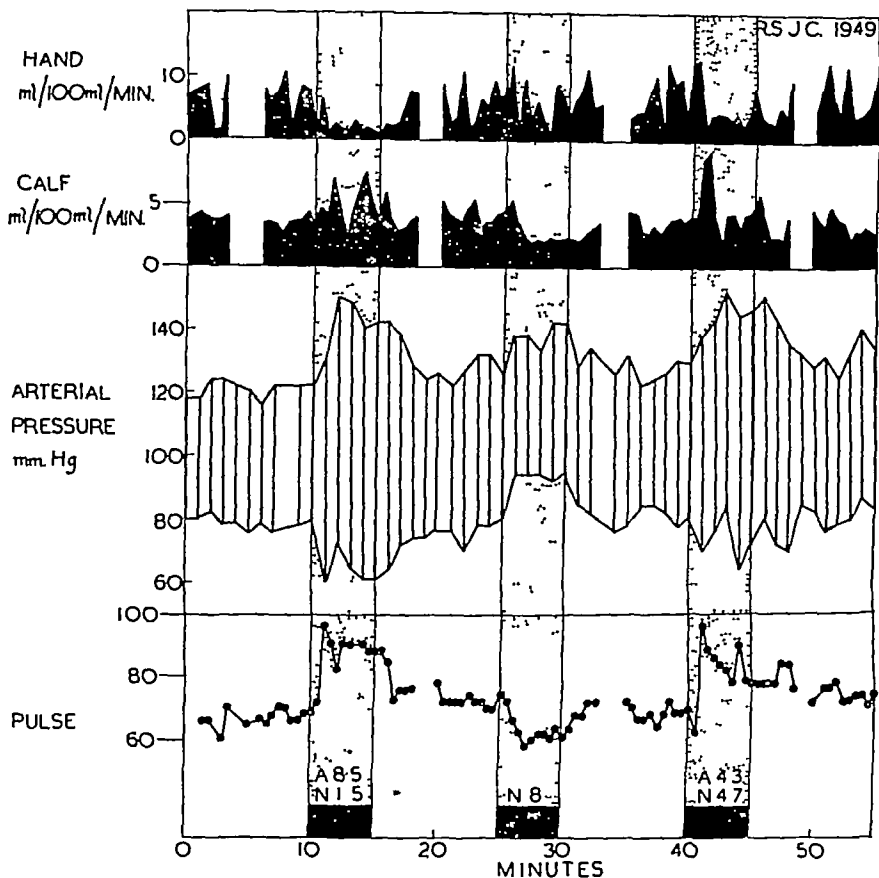
Fig 1   The results of one of the twelve experiments reported in this paper   The stippled columns indicate the periods of drug infusion.   Dosage is given in micrograms of base per minute, A, of l adrenaline, B, of l noradrenaline

The results of these experiments were averaged   (Figs 2-5)   Every point on the graphs is the average of 6 observations   In arriving at these averages it was necessary to deal with missed or doubtful observations, of which there were 48 in a total of 2,628   To omit them and hence to calculate some of the averages from less than six observations might have introduced a large error   It was decided, therefore, to assign to a missing observation during a base line period before a drug infusion started the

average value of the other observations during that period, and to a missing observation during or after a drug infusion, a value midway between that of adjacent observations

The results of one experiment on a 6th subject have been discarded from this report   This subject developed a very high blood pressure, and alarming symptoms when a mixture containing mainly noradrenaline was infused (2) He has since been tested with graded doses, and the original dose has been repeated, producing a response within the normal range   We do not think there can have been an error of dosage, but we considered it better to omit this anomalous result

The heart rate   Adrenaline, containing 12-18% noradrenaline, caused an increase in heart rate reaching a peak of about 25 beats per min 1 5 min after the infusion started, followed by a sustained increase of about 15 beats per min (Fig 2)   Noradrenaline caused a sustained fall of about 15 beats per min   The adrenaline effect predominated when a mixture of equal parts was given, and the effects balanced at adrenaline-noradrenaline ratios between 1 3 and 1 8

The arterial blood pressure   Adrenaline raised the systolic pressure by about 15 mm Hg and lowered the diastolic pressure by 5-10 mm Hg (Fig 3) Noradrenaline raised the systolic pressure by about 10 mm Hg and the diastolic pressure by about 15 mm Hg   The diastolic pressure was unchanged when a mixture of equal parts was given

The blood flow through the calf   Adrenaline caused a large transient increase in calf blood flow which reached a maximum about 1 5 min after the infusion started, followed by a smaller secondary increase (Fig 4) Noradrenaline decreased the blood flow   The adrenaline effect predominated when the mixture contained equal parts, and with an adrenaline-noradrenaline ratio of 1 3, but the noradrenaline effect predominated with a ratio of 1 8

The blood flow through the hand   Adrenaline and noradrenaline both reduced the blood flow through the hand (Fig 5)   The reduction was greater with adrenaline   Intermediate ratios all reduced the hand flow, but it is difficult to say from the results whether the effect of one or other substance predominated

Discussion

Our results with l-adrenaline (containing 12-18% of noradrenaline) and l-noradrenaline confirm those of previous workers (1, 4, 8, 12)

A mixture of equal parts of adrenaline and noradrenaline has been infused by Goldenberg *et al* (8), but the mixture followed the infusion of noradrenaline alone, so that it is difficult to compare their results with our own

PULSE RATE   AVERAGE OF 6 EXPERIMENTS
5 MINUTE INFUSIONS

Fig 2

CALF BLOOD FLOW   ml/100ml/MIN.
5 MINUTE INFUSIONS   AVERAGE OF 6 EXPERIMENTS
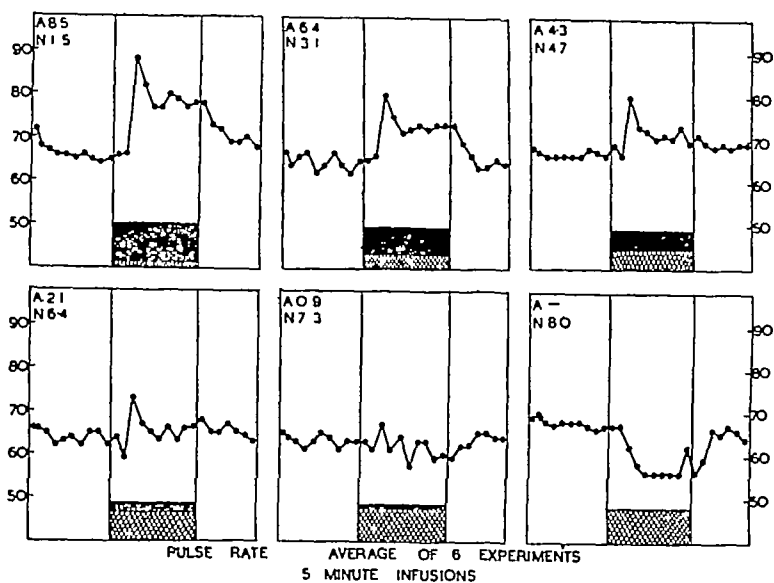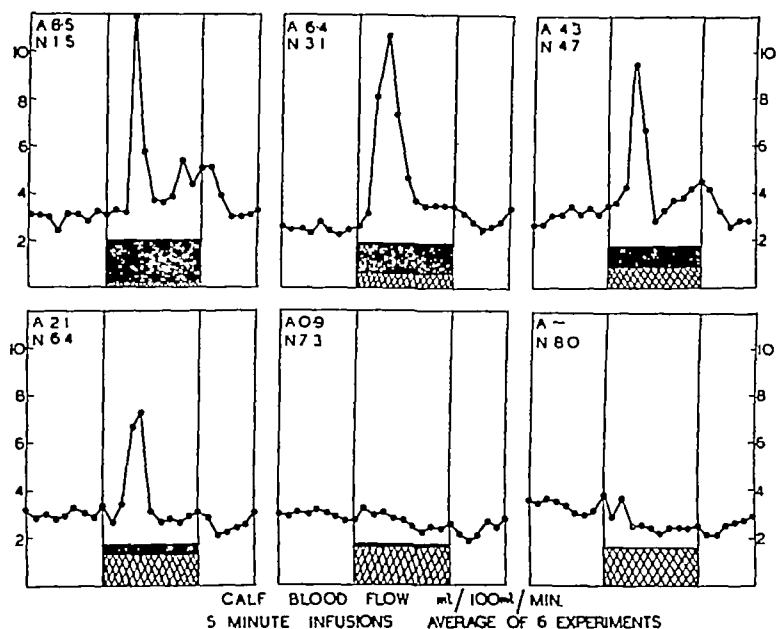
Fig 4

Figs 2 5   The average results of infusion of various mixtures of 1 adrenaline and 1 noradrenaline   D
period of infusion, the solid black indicating the dose of 1-adrenal

BLOOD PRESSURE   AVERAGES OF 6   EXPERIMENTS
5 MINUTE INFUSIONS

Fig 3

HAND BLOOD FLOW ml/100 ml/MIN.
5 MINUTE INFUSIONS
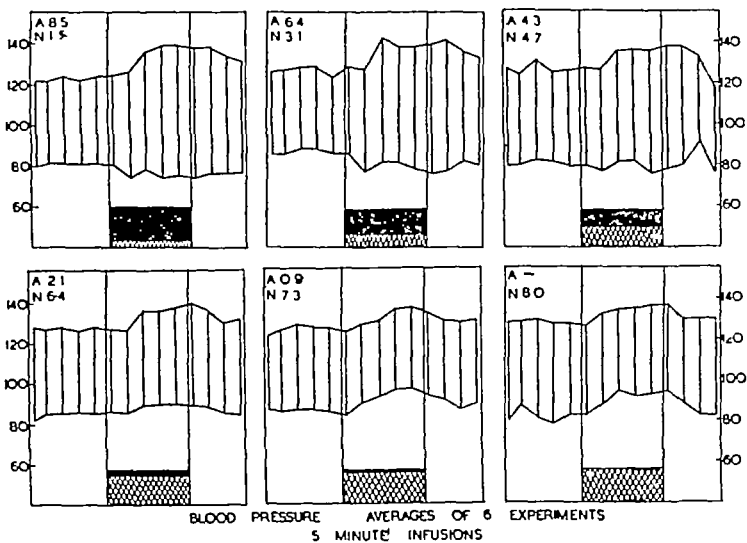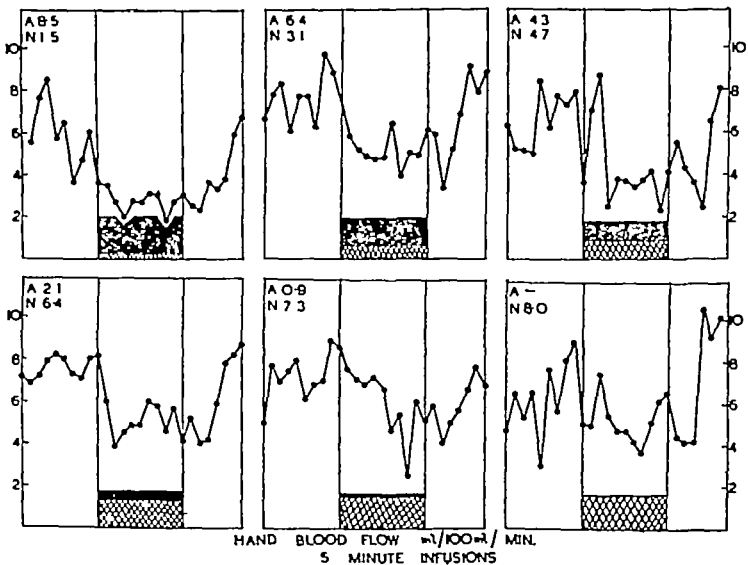
Fig 5

given in micrograms of base per minute A, of 1 adrenaline, N, of 1 noradrenaline   The blocks represent the
the cross hatching the dose of 1 noradrenaline

We made our infusion intravenously to simulate the possible release of mixtures of the substances from the adrenal glands This tested the overall effect of the mixtures, and no conclusions can be drawn from our results as to the direct effects of the mixtures on the heart or the blood vessels of the skin or muscles

Our results show an even gradation of response with the different mixtures A small quantity of either substance fails to block the effects of the other Generally, however, the adrenaline effects predominate in mixtures of equal parts

Summary

1 A series of mixtures of l-adrenaline and l-noradrenaline have been infused intravenously into normal human subjects Observations have been made on the heart rate, the arterial blood pressure, and the blood flow through the hand and the calf

2 With mixtures of equal parts the adrenaline effect predominates

3 Opposing effects balance with mixtures containing 3 or 8 parts of noradrenaline for each part of adrenaline

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POSTURAL CHANGES IN THE PERIPHERAL BLOOD-FLOW OF NORMAL SUBJECTS WITH OBSERVATIONS ON VASOVAGAL FAINTING REACTIONS AS A RESULT OF TILTING, THE LORDOTIC POSTURE, PREGNANCY AND SPINAL ANÆSTHESIA

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CHANGE from the horizontal to the erect posture in man is accompanied by little or no change in blood pressure Cardiac output is, however, significantly decreased in quiet standing in spite of some acceleration of the heart (13) Vasoconstriction must therefore occur to maintain the blood pressure Others have presented evidence that constriction takes place in the vessels of the skin of both trunk and extremities when the erect posture is assumed (18, 14, 12) This paper presents results obtained from the study of forearm blood-flow first of normal subjects tipped into the erect posture and, secondly, of some subjects in whom the blood pressure was not maintained in response to postural change

Methods

The majority of subjects were studied on a tipping table with the feet supported Forearm blood-flow was measured with the venous occlusion plethysmograph (1) which was arranged so that the pressure in the veins of the arm was similar in the supine and the erect posture Cardiac catheterisation was performed and cardiac output measured by methods previously described (13) Intra-arterial and intra-cardiac pressures were recorded photographically using a condenser manometer (9, 10) It is difficult to obtain strictly comparable records of right auricular pressure when posture is changed In the subjects in which auricular pressure was measured, the centre of the right auricle was judged on an X-ray tipping couch in the horizontal and the erect positions and marked on the chest wall before the observations were commenced With the subject on the tipping table, the manometer was then placed at the level of the corresponding mark

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Results

Normal subjects The results obtained in twelve normal subjects are shown in Table I, and an example in Figure 1 On tilting the subjects from the supine to the erect feet-down position the forearm flow decreased sharply, the maximum decrease occurring immediately on tipping Thereafter, for

TABLE I

Normal subjects

Case No	Sex Age	Forearm flow cc per 100 cc per min			Systolic blood pressure supine	Systolic blood pressure upright	Heart rate supine	Heart rate upright
		Supine	Upright 10 to 60 secs	Upright 5 to 10 mins				
1	M 15½	4 5	3 5	4 0	100	108	64	96
2	M 34	2 6	2 0	2 2	114	104	64	72
3	M 24	3 0	1 0	1 3	124	110	75	90
4	M 35	4 0	2 4	3 5	125	130	69	84
5	M 27	4 4	1 9	2 2	132	136	76	84
		3 2	0 6	2 1	135	128	64	84
6	M 30	3 4	1 9	3 5	110	110	68	92
7	M 41	2 4	1 8	2 2	120	115	64	80
8	M 35	5 0	3 5	3 6	115	115	70	84
9	M 61	2 5	1 3	2 0	150	150	84	100
10	F 29	2 6	0 5	1 0	120	122	72	90
11	F 13	3 6	1 6	1 8	105	104	72	100
12	M 16	4 2	1 6	2 5	120	110	75	100

the next few minutes, the flow increased and in a few subjects approximated to the original supine flow In the majority of subjects, however, the flow in the erect posture remained at a lower level than that observed in the supine position On returning the subjects to the supine position the flow

in the forearm increased to the previous resting level Momentary constriction occurred occasionally in anxious or labile subjects on tipping backwards to the supine position, but this could usually be eliminated by a preliminary practice
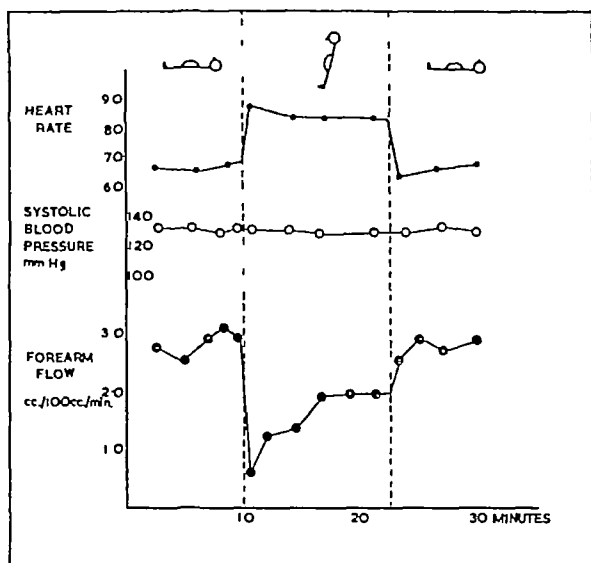
Fig 1 Normal subject In the erect posture forearm flow decreases, blood pressure is maintained

Continuous blood pressure recordings were taken from a needle in the brachial artery In general, little change in the mean pressure was observed although pulse pressure was decreased in the erect posture and the heart rate was increased In some subjects the blood pressure fell slightly on tipping (Fig 2), in others it rose (Fig 3), and in several fluctuated within a narrow range Slowing of the heart with increased pulse pressure was observed in every subject when restored to the supine from the erect posture

Continuous records of right auricular pressure reveal an immediate pressure fall when the subject was tilted into the erect posture (Fig 4) The difficulty of obtaining comparable readings in the two positions may have introduced an error of 1-2 cm saline, but from the records taken it would appear that the average decrease in mean right auricular pressure on assuming the erect posture was 7 cm of saline

Sympathectomised subjects In two subjects in whom cervical sympathectomy had been performed no change in forearm blood-flow was observed on tipping from the supine to the erect posture (Fig 5) These

observations would appear to indicate that the vasoconstriction observed in the normal subject was a vasomotor reflex, with the efferent pathway through the vasomotor nerves
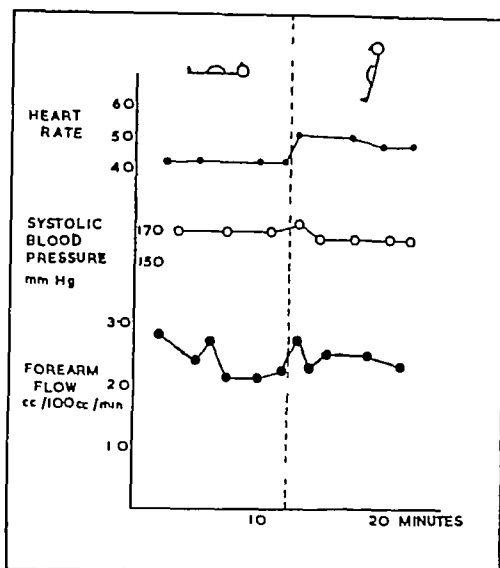
Fig 5 Subject with sympathectomised forearm There is no change in flow on tilting into the erect posture

Vasovagal fainting Some normal subjects are unable to maintain the erect posture on a tipping table without feeling faint These subjects may give a history of fainting easily all their lives An example is shown in Fig 6 On tipping into the erect posture this subject showed the normal constriction in the forearm After a time, however, the blood pressure began to fall and the forearm flow increased in parallel, the maximum increase in flow occurring at the time of the lowest blood pressure, at this point too, the heart rate was slower than it had been previously On tipping back into the supine position blood-flow and blood pressure returned to previous levels When again tipped upright after the normal initial constriction he again showed the same phenomena The final fall of blood pressure was preceded by a short period in which the blood pressure fell slightly and the flow increased slightly This we have observed in others It would appear that when muscle vasodilatation commenced, accompanied by a fall in blood pressure, a subject could, consciously or unconsciously by voluntary or involuntary muscular movements, reverse the process, causing vasoconstriction and a rise in blood pressure However, sooner or later, full muscle vasodilatation occurred, the blood pressure fell to low levels and was accompanied by all the other phenomena of the vasovagal faint—bradycardia, sweating, pallor, over-breathing, unconsciousness and

occasionally epileptiform fits   Blood pressure and forearm flow were restored immediately on tipping back into the supine posture though pallor and some bradycardia remained
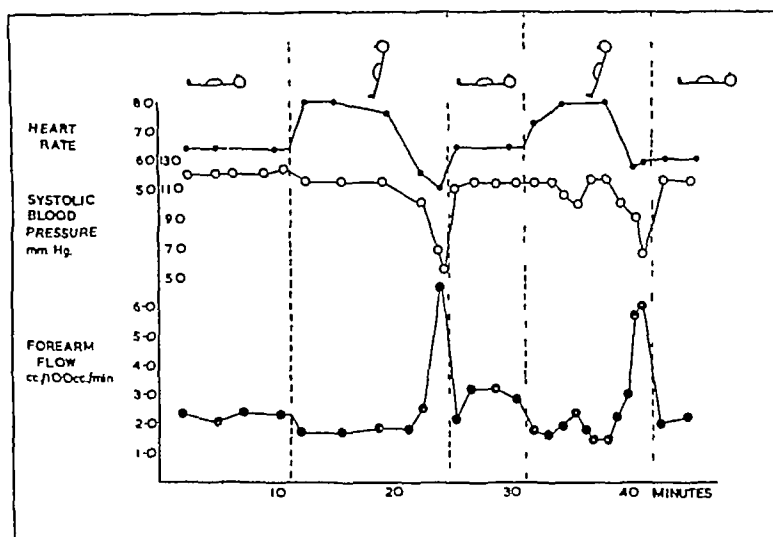
Fig 6   Normal subject, showing muscle vasodilatation, fall of blood pressure and bradycardia in the erect posture (*see* text)

Fainting in the erect lordotic position   Prolonged standing in the erect lordotic position will frequently produce fainting, especially in young subjects The mechanism of this form of fainting is illustrated by case R N (Fig 7) He complained of fainting in queues, although a long distance cyclist of good physique   Standing in the kyphotic position with one leg raised on a stool, he showed normal constriction in the forearm, and blood pressure was maintained   On assuming the lordotic posture with the feet together the heart rate increased steadily to reach 180 per minute, forearm flow decreased further and blood pressure was initially maintained   Suddenly, however, there was an acute fall in blood pressure, forearm flow increased, the heart rate slowed from 180 to 40, and the subject became unconscious

The mechanism of this type of faint would seem to be as follows   In the kyphotic posture, right auricular pressure, inferior vena caval pressure and cardiac output were decreased as in the normal subject   Right auricular pressure and inferior vena caval pressure were measured by a catheter and a saline manometer   In the lordotic posture, inferior vena caval pressure rose, while right auricular pressure and cardiac output fell further   It is interesting to note that the cardiac output with a heart rate of 180 per minute was only 2½ litres per minute   The lordotic posture therefore resulted in an obstruction of the inferior vena cava, probably at the level of the

diaphragm with considerable pooling of blood in the lower part of the body Measurements of inferior vena caval pressure by Bull (4) and by us in other normal subjects have shown a rise in the lordotic posture   Subjects with a great rise of pressure exhibit postural albuminuria (4) as did case R N and the mechanism producing the two conditions would appear to be the same

Four other subjects have shown similar vasovagal reactions and in six young subjects some constriction in the forearm vessels has been induced by the extreme lordotic posture
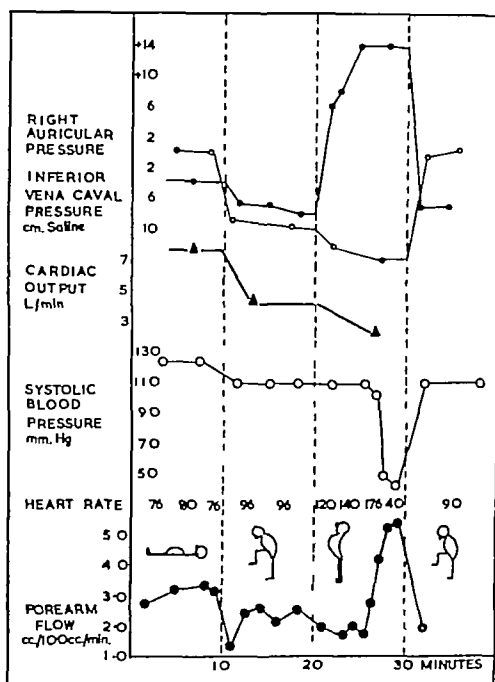
Fig 7   Normal subject, showing muscle vasodilatation, fall of blood pressure and bradycardia with a rise of inferior vena caval pressure and a fall in right auricular pressure in the erect lordotic position (*see* text)

Fainting in pregnant women   A certain proportion of pregnant women will faint if maintained in the strictly supine position (11) and the mechanism is probably similar to that described in the last section

Figure 8 shows a typical result in a subject who had fainted in a dentist's chair   Blood pressure, heart rate and forearm blood-flow were normal when lying on the side   When the strictly supine posture was assumed, the blood pressure fell, heart rate increased and forearm blood-flow decreased   On turning to the side again, the circulation was restored to normal   No attempt was made to induce a faint with muscle vasodilatation in this subject as it was felt that too great a fall of blood pressure might be harmful

In 1924 Runge (17) observed that the venous pressure was higher in the leg than in the arm veins in late pregnancy and that the leg vein pressure fell after delivery   When the venous pressure in the legs of a subject in late pregnancy was measured with a condenser manometer, it was found that the pressure rose 7 5 cms in the supine position, and fell on turning slightly to one side
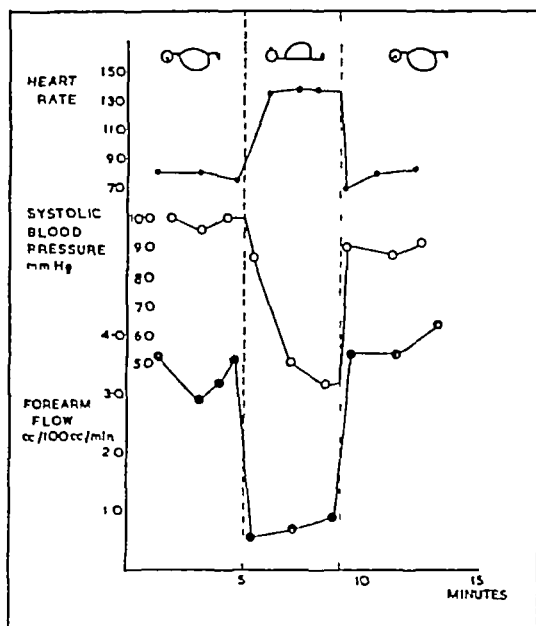
Fig 8   Normal woman, 38 weeks pregnant, showing decrease in forearm flow and blood pressure with tachycardia in the strictly supine position (*see* text)

In late pregnancy, therefore, the uterus may obstruct the veins of the abdomen when the subject is in the strictly supine position, causing a rise of venous pressure caudally, and a fall in pressure in the right auricle , the exact point of venous obstruction has not as yet been determined   Cardiac output has been found by Palmer and Walker (15) to decrease *pari passu* with the falling right auricular pressure in cases of late pregnancy maintained in the supine position

Normal women or women in early pregnancy showed no change in forearm blood-flow or leg venous pressure with similar postural changes

Fainting after spinal anæsthesia   In the supine position, blood pressure, right auricular pressure and cardiac output are decreased following the administration of a spinal anæsthetic (16)   All subjects when tipped into a more upright posture showed a great fall of blood pressure and bradycardia

Forearm blood-flow was studied in one case   The sequence of events is shown in Fig 9   After the spinal anæsthetic, blood pressure and forearm flow were decreased in the supine position   On tipping to 30 degrees from the horizontal, forearm flow at first decreased, then showed a sudden increase, at which time there was an acute fall in blood pressure, accompanied by the typical phenomena of the vasovagal faint
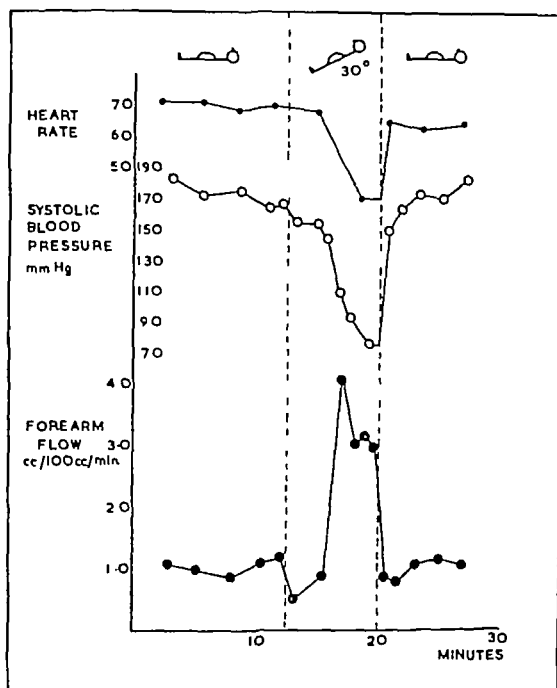
Fig 9   Hypertensive subject   A spinal anæsthetic was given before the observations charted above were made   Muscle vasodilatation, fall of blood pressure and bradycardia follow tipping 30 degrees (*see* text)

Spinal anæsthesia would appear to produce not only arteriolar dilatation in the anæsthetized portion of the body, but also venous dilatation, since right auricular pressure falls (16)   Vasoconstrictor tone is maintained, or perhaps even increased, in the unaffected part of the body, though blood-flow falls off with a falling blood pressure   When the subject is tipped into a more erect posture, there is a further fall in the right auricular pressure, accompanied by a further fall in blood pressure and decrease in forearm blood-flow   The constrictor tone in the muscle vessels of the unanæsthetized part of the body, hitherto maintained, is suddenly abolished , vasodilatation occurs, accompanied by an acute fall of blood pressure   Hypotension after spinal anæsthesia depends therefore on a number of factors , initially loss of arteriolar tone in the anæsthetized region is accompanied by a decrease

in cardiac output secondary to a decreased filling pressure of the heart, finally, muscle vasodilatation in the unanæsthetized portion of the body reduces the blood pressure to very low levels
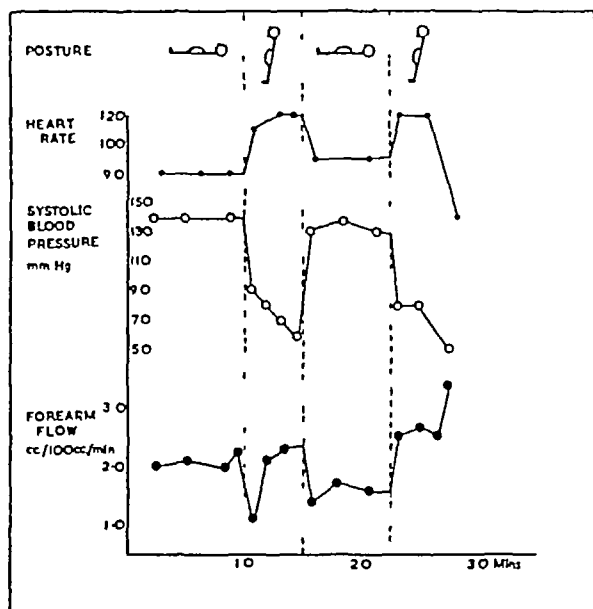
Fig 10   Syphilitic paraplegia, showing muscle vasodilatation, fall of blood pressure and, in the second observation, bradycardia in the erect posture

Fainting in nervous diseases   Diseases of the spinal cord may occasionally give rise to postural fainting attacks (7, 6)   A patient with meningovascular syphilis who showed this phenomenon was investigated and the results are shown in Fig 10   The acute fall of blood pressure in this subject was accompanied by vasodilatation in the forearm   Decreased arteriolar and venous tone in the lower extremities may have played a part in this form of acute hypotension, as in the case of the subjects with spinal anæsthesia   On another occasion when ambient temperature was cooler a severe fall in blood pressure could not be produced (Fig 11)

Discussion

When a normal subject is placed in the erect posture the veins of the lower part of the body dilate, the pressure in them increases and the filling pressure of the heart falls   Cardiac output decreases in spite of cardiac acceleration, yet blood pressure is maintained   A normal subject bled in the supine position shows a similar fall in right auricular pressure, and cardiac output, with again a cardiac acceleration and an unchanged blood pressure (13)   Tipping into the erect posture may thus be regarded as a

functional hæmorrhage   Vasoconstriction in muscle blood vessels has been shown to occur in every case when tipped into the feet down position and that this is a reflex phenomenon has been shown by its absence in the sympathectomised arm   It is concluded therefore, that the blood vessels in muscle, as well as those in the skin and other organs, play a part in maintaining the blood pressure in the erect posture   Previous studies of
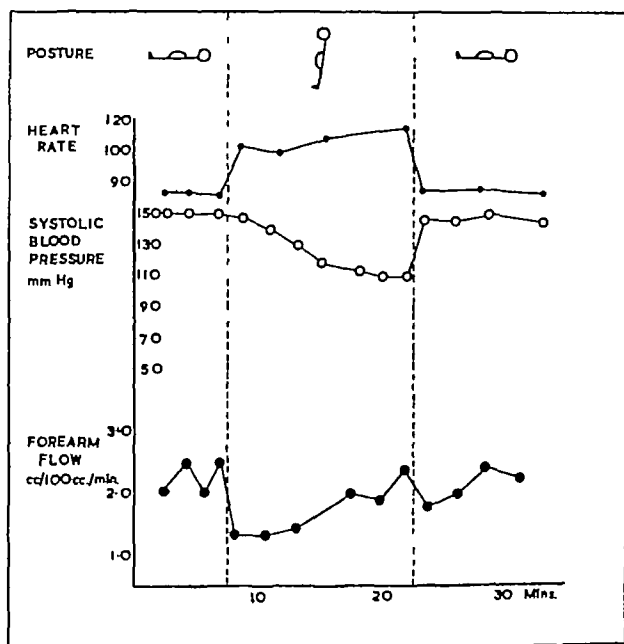
Fig 11   Same subject as Fig 10   On an occasion when ambient temperature was cooler conspicuous muscle vasodilatation and fall of blood pressure did not occur

forearm blood-flow in normal subjects during bleeding, however, have shown no constant or significant changes provided that fainting did not occur (2, 5)   The vasoconstriction occurring in muscle following postural change may perhaps result from the equivalent of a greater rate and amount of hæmorrhage   Continuous records of right auricular pressure have shown this to be considerable immediately on tipping to the feet-down position

Vasoconstriction was also observed to occur in the forearm when the great veins of the abdomen were obstructed in cases of late pregnancy and in the lordotic posture , the decrease in circulating blood volume in both types of case must also have been sudden and considerable

If bleeding is continued, the normal subject shows an acute fall of blood pressure and a sudden slowing of the heart , at this stage it has been shown that vasodilatation occurs in muscle vessels (3) and that this is a reflex, the efferent pathway of which is mediated by the vasomotor nerves (2) Apparently normal subjects maintained in the erect posture on a tilting

table and susceptible young subjects kept in the lordotic posture show a vasovagal phenomenon indistinguishable from that produced by bleeding There is a profound fall in blood pressure with considerable cardiac slowing, and the postural vasoconstriction gives way to vasodilatation in forearm blood vessels In spinal anæsthesia and disease of the spinal cord, the attacks differ solely in the fact that the blood pressure has already been reduced from other causes Fainting in late pregnancy is undoubtedly of the same type

One case of psychological fainting observed fortuitously by us also showed the characteristic vasovagal syndrome, the acute fall in blood pressure being accompanied by an increase in forearm blood-flow, and a similar observation has also been made by Greenfield (8) "Fainting" in all types of case reported here, therefore, was found to be of vasovagal type, and there are no grounds for believing that the mechanism is not identical in all groups

SUMMARY

1 When normal subjects were tipped from the supine to the erect posture there was a decrease in right auricular pressure and cardiac output and a decrease in the forearm flow The decrease in forearm flow did not occur in sympathectomised forearms Blood pressure was maintained

2 Some normal subjects, with a history of fainting easily, showed after a period in the erect posture vasodilatation in the forearm which paralleled the fall in blood pressure

3 Some young normal subjects in the erect lordotic position showed a further fall of right auricular pressure but a rise of inferior vena caval pressure In spite of extreme tachycardia cardiac output fell to low levels Initial constriction in the forearm and maintenance of blood pressure was followed by forearm vasodilatation, fall of blood pressure and bradycardia The kyphotic position restored the circulation

4 In late pregnancy, the strict supine position resulted in a fall of auricular pressure and cardiac output, a rise in leg vein pressure and constriction in the forearm The circulation was restored to normal by turning slightly to one side

It is suggested that the uterus may cause obstruction to the great veins inside the abdomen

5 Following spinal anæsthesia tipping into the semi-erect posture caused initial further constriction in the forearm followed by dilatation and a great fall in blood pressure

A case of spinal cord syphilis showed similar responses

6 These fainting reactions are similar to those observed on bleeding normal supine subjects It is suggested that they result from a greater amount of blood collecting in the veins of the lower half of the body and a consequent fall in right auricular pressure

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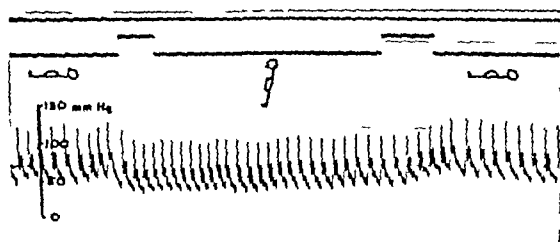
Fig 2   Normal subject   Arterial blood pressure in brachial artery   At the first signal the
subject is tipped into the erect posture   and at the second signal is tipped back into the
supine position   This subject shows exceptional fall in blood pressure in the erect posture
Time marker in seconds
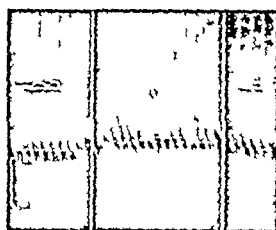
Fig 3   Normal subject   Continuous blood pressure record from the brachial artery   Vertical
white lines are signals indicating   first, tipping into the erect posture   and secondly, tipping
back into the supine position   In this subject there was a slight rise in blood pressure
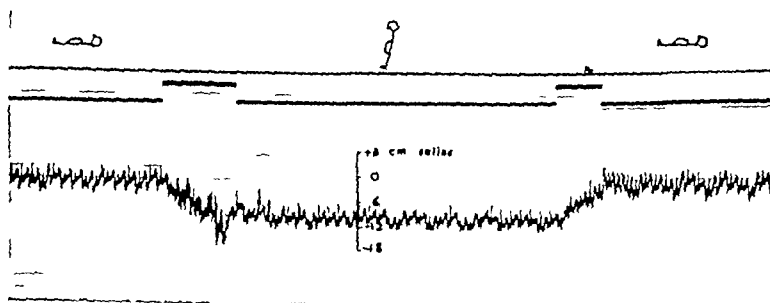
Fig 4   Normal subject   Right auricular pressure tracing   Zero represents centre of auricle
Auricular pressure falls in the erect posture

POSTURAL CHANGES IN PERIPHERAL BLOOD FLOW IN CASES WITH LEFT HEART FAILURE

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CHANGE of posture is a simple method of altering the venous pressure and has the advantage of rapid reversal   Normal subjects showed a decreased forearm flow in the erect posture as compared with the supine position, and this decrease was mediated through the vasomotor nerves since there was no change in sympathectomised arms (2)   This paper reports the effects of postural changes on forearm flow in cases with hypertensive, ischæmic, and valvular heart disease and anæmia

Methods

Forearm flow was measured by plethysmography (1) and right auricular pressure with a condenser manometer (3)   Some subjects were investigated on a tipping table   The more severely ill patients were studied in bed with the body propped up to 45 degrees when the bed was horizontal   Forty centimetre blocks were placed alternately under the wheels at the head and the foot of the bed   In the " feet–down " position this resulted in the body being about 60 degrees from the horizontal and the legs inclined downwards at 15 degrees   In the " feet–up " position the body was about 30 degrees above the horizontal, and the legs raised about 15 degrees

The subjects of this study were twelve patients who gave a history of attacks of acute dyspnœa and all had had such an attack of minor or major degree within forty-eight hours of the first observations being made   Six other patients with hypertensive or aortic disease and enlarged left ventricles who did not complain of postural dyspnœa were also investigated

Results

Results are shown in Table I and examples in Figs 1 and 2   In all the patients with left heart failure the forearm flow decreased in the " feet-up " position showing a reversal of the changes produced by similar postural

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TABLE I

Cases with postural dyspnœa

Case No	Sex and age	Diagnosis	Venous pressure Cm Saline above S.A	Method of tipping	Forearm flow cc/100cc/min			Systolic blood pressure feet up	Systolic blood pressure feet down	Heart rate feet up	Heart rate feet down
					feet up	feet down 1 min	feet down 5 10 mins				
1	M 50	Aortic incompetence	−5	T back to 30°	2·5	3·4	4·4	135	140	75	80
2	M 54	Aortic incompetence	+20	B	1·1	3·0	3·1	138	134	84	84
3	M 75	Ischaemic heart disease	+6	B	0·6	1·4	1·4	120	100	92	92
4	M 71	Hypertension	+4	B	1·0	1·8	1·7				
5	M 60	Ischaemic heart disease	+25	B	0·3	0·8	0·7				
6	M 35	Haematemesis	+4	B	2·8	4·0	3·8	88	84	94	96
7	M 58	Addisonian anaemia	+4	B	1·4	3·4	3·3	110	95	78	80
8	F 63	Ischaemic heart disease	+16	B	0·6	0·9	1·1				
9	M 69	Hypertension	+1	B	0·6	1·7	1·6	220	105	94	
10	M 68	Hypertension	+3	B	0·7	1·3	1·4	155	145	90	80
11	M 61	Hypertension	−4	B	0·9	2·5	2·8	180	108	100	100
12	M 73	Ischaemic heart disease	+6	B	1·7	3·6	3·8				

T = tipping table   B = bed and blocks (*see* methods)

change in normal subjects or in patients with heart disease but no attacks of dyspnœa (Table II) In many patients the procedure was repeated once or more with similar results
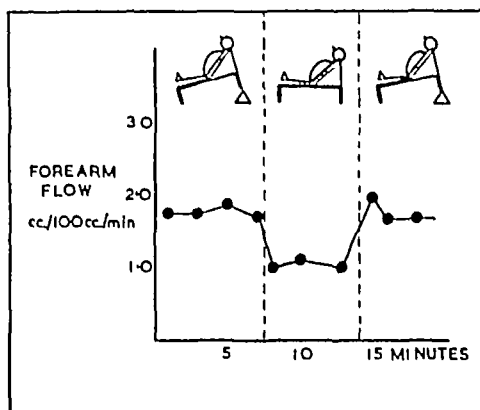
Fig 1 Case 4 Hypertensive heart disease
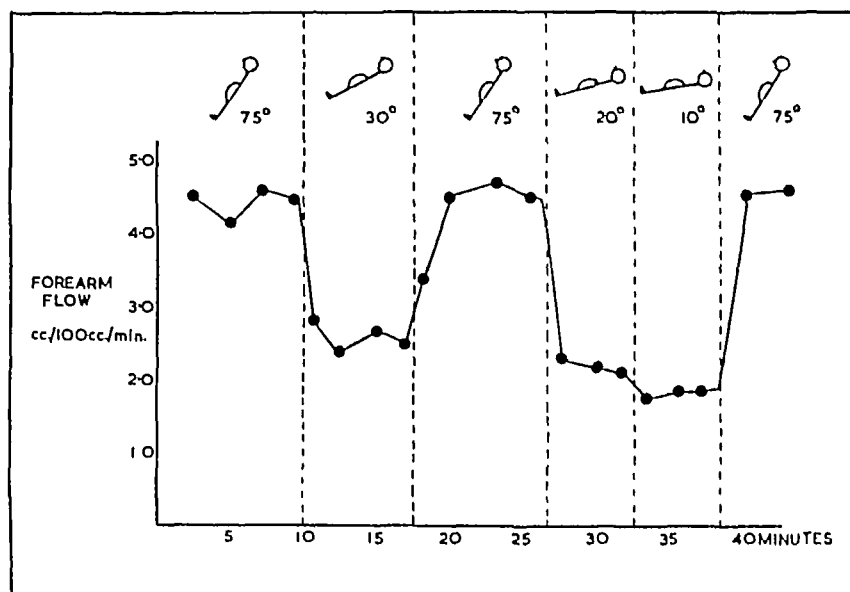
Fig 2 Case 1 Aortic incompetence

As will be seen in Table I the initial venous pressure was variable and in two cases apparently normal The postural change in right auricular pressure is illustrated in Fig 3 No significant change in blood pressure was observed in Cases 1, 2 and 6 Cases 3, 7, 9, 10 and 11 showed a rise in

TABLE II

Cases without postural dyspnœa

Case No	Sex and age	Diagnosis	Venous pressure	Method of tipping	Forearm flow cc/100cc/min			Systolic blood pressure supine	Systolic blood pressure erect	Heart rate supine	Heart rate erect
					supine	erect 1 min	erect 5 10 mins				
13	F 65	Hypertension	Normal	T	5 0	1 7	3 3	260	235	80	108
14	M 52	Hypertension	Normal	T	2 4	0 0	2 2	204	192	08	70
15	M 45	Hypertension	Normal	T	3 1	0 0	1 6	188	190	74	90
16	M 60	A I , A S	Normal	T	2 4	1 1	1 4				
17	M 39	Hypertension	Normal	T	2 8	1 2	1 7	235	182	02	104
18	M 34	A I , A S	Normal	T	2 3	0 5	0 9				

T = tipping table

the "feet-up" position   The heart rate showed little variation   The six patients with hypertension or aortic valvular disease but no postural dyspnœa showed the same response as normal subjects (2)
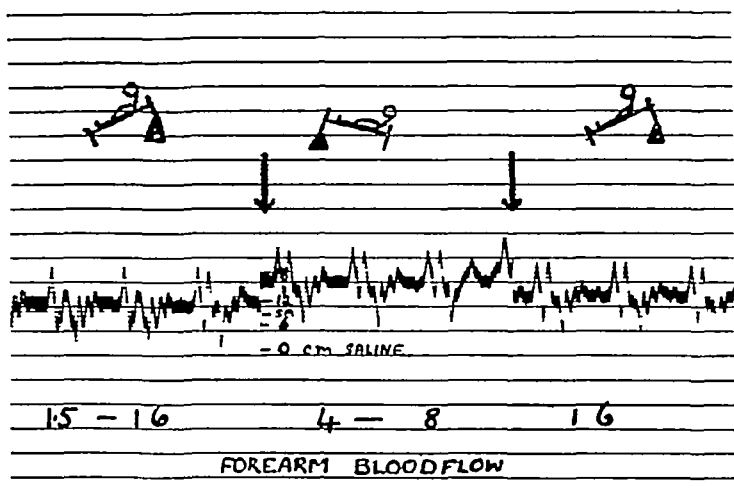
Fig 3 Case 9   Hypertensive heart disease   Right auricular pressure curve, showing a rise in the feet-up position.

Effect of acute nerve block   In two patients the median, radial and ulnar nerves were successfully blocked with 4% procaine   This procedure abolished the postural change in forearm flow (Fig 4)

Effects of treatment   As might be expected successful treatment of cases of hypertensive and valvular heart disease resulted in a reversal of the postural flow changes to the normal pattern   In severe anæmia (9, 10) or prolonged recurrent hæmorrhage (6) small transfusions may precipitate attacks of left heart failure   Fig 5 shows the effect of postural change in a young male with recurrent hæmatemesis   There was constriction in the "feet-up" position   Nineteen days later following transfusion and iron administration the postural response was normal

Effects of high initial arm vein pressure   Since a number of cases of left heart failure had high initial venous pressure, it was thought desirable to study cases where the arm vein and jugular pressures were increased but cardiac function and right auricular pressure were normal   Two cases of superior vena caval block were investigated (Fig 6)   The venous pressure in the arms was greatly increased and the response to postural change was the same as in normal subjects   In one of these cases a catheter was passed beyond the block in the superior vena cava and the pressure in the right

auricle was measured and found to be normal In the other case the function of the heart was normal and it is reasonable to assume that right auricular pressure was also normal
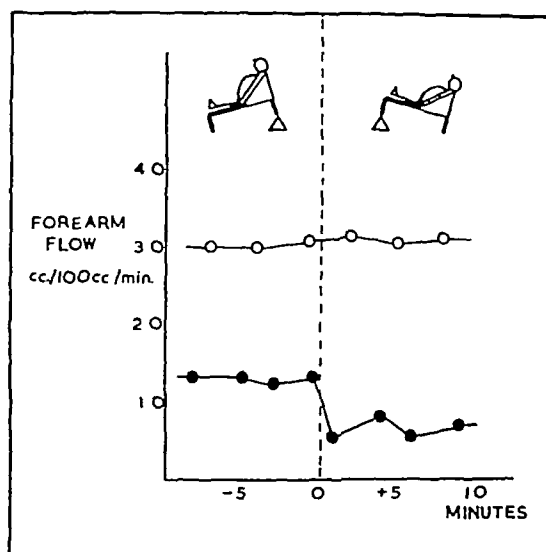
Fig 4 Case 9 Hypertensive heart disease Black circles show the response to posture in a normally innervated forearm White circles show the response in the same forearm when the radial, median and ulnar nerves were blocked with 4% procaine
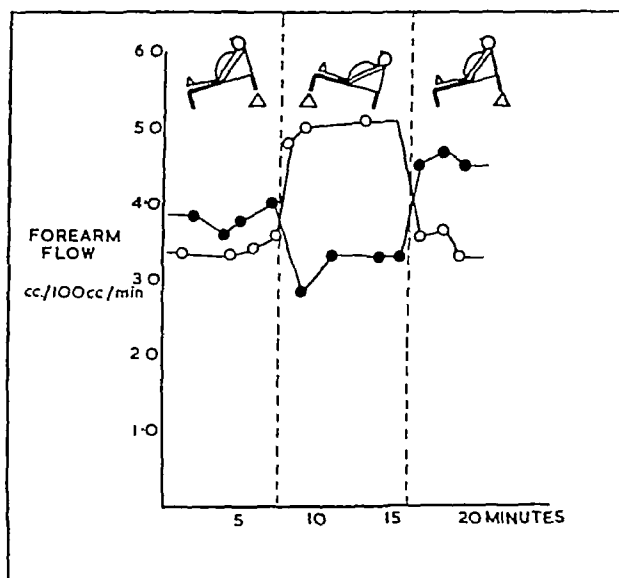
Fig 5 Hæmatemesis Black circles show postural flow response on admission when venous pressure was 4 cms above sternal angle , systolic blood pressure 85 mm.Hg and hæmoglobin 45% White circles show flow response 19 days later when venous pressure was normal, systolic blood pressure 118 mm Hg and hæmoglobin 81%

Discussion

The results of postural change in cases with left heart failure are similar to those obtained by changing venous pressure by other methods

In cases of severe chronic congestive heart failure from hypertensive, ischæmic, or valvular heart disease forearm flow was usually diminished When right auricular pressure was lowered by venesection the low forearm flow increased in spite of a fall of blood pressure (7)
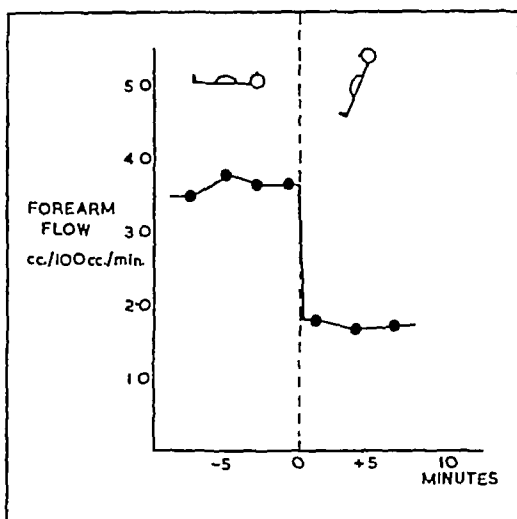
Fig 6   Superior vena caval obstruction   The venous pressure was 36 5 cms above the ante cubital vein in both positions   The postural flow response was normal

Spontaneous attacks of acute left heart failure in similar cases were associated with a rise in right auricular pressure, the blood pressure increased and there was constriction in the forearm and hand (8)   Similar changes were produced in normal subjects by giving rapid intravenous infusions so that right auricular pressure was raised to high levels   The blood pressure rose and there was constriction in the forearm   Rather smaller infusions produced the same result in subjects suffering from malnutrition (4, 5), myxœdema (8), or severe anæmia (9, 10)   The cases with superior vena caval block had even higher pressures in arm and jugular veins but normal auricular pressure   The normal forearm flow and normal response to posture in these cases indicate that constriction does not result from a rise of pressure distal to the superior vena cava

The total evidence to date suggests that a rise of central venous pressure results in constriction of forearm vessels and that while great changes of right auricular pressure are needed in normal subjects, smaller changes are

effective in patients with disease of the chambers of the left heart   These effects would appear to be a reflex of which the efferent pathways are the vasomotor nerves

In practice measurement of postural change in forearm blood flow affords a simple and rapid method of determining the presence of potential left heart failure or of following the effects of treatment and other procedures

SUMMARY

1   The effect of posture on forearm flow was measured in twelve cases of hypertensive, ischaemic, valvular heart disease and severe anæmia with attacks of left heart failure   In the "feet-down" position forearm flow was greater than in the "feet-up" position, a reversal of the findings in normal subjects and cases of hypertensive and aortic valvular heart disease without left heart failure

2   No change in blood flow with postural change was found when the nerves to the forearm were blocked by 4% procaine

3   Two cases of superior vena caval block with high venous pressures in the upper part of the body but normal right auricular pressures showed the same postural response as normal subjects

4   In cases with left heart failure responding to treatment, the postural flow response changed to normal

5   The effect of lowering venous pressure by change of posture in cases with left heart failure was similar to the effects of lowering venous pressure by venesection

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VENOUS PRESSURE MEASUREMENT IN THE FOOT IN EXERCISE AS AN AID TO INVESTIGATION OF VENOUS DISEASE IN THE LEG

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UNTIL the recent revival of interest in the alleviation of the pain, ulceration and other results of venous lesions in the legs, the only radical treatment offered was ligation or excision of varicose, incompetent superficial veins Within the past few years, however, the disabling sequelæ of disorders of the deep veins have been treated by ligation of the superficial femoral (5) and the popliteal veins (3) It is particularly in the latter procedures that we have been interested

The available methods for investigating these cases have been based on the responses of visible superficial veins to changes of posture and activity with and without a tourniquet compressing the superficial veins, and on X-ray photography of the veins following injection into them of a radiopaque substance Linton has measured the intravenous pressure in the femoral vein before and after occluding it, with the patient horizontal, so as to determine whether it should, or should not, be tied

In order to measure the hydrostatic effects of venous disease, and to estimate accurately the response to treatment, we have studied the fluctuations in venous pressures in the legs under varying conditions Since the maximal effects of venous disease are seen in the lower leg and the maximal pressure variations occur in the foot it is here that pressure measurements are most likely to be of value

Recently Pollack and others (6) have applied similar methods to the same idea and have investigated venous pressures in the foot in cases of varicose veins secondary to deep venous thrombosis

The present paper, begun before their findings were published, is concerned with the observations on 27 patients

Venous hydrostatics in the leg at rest and during exercise

At rest, whether in the horizontal or vertical position, blood is returned from the leg by the arterial vis-a-tergo which propels the blood towards the

heart through passive venous channels It is clear, therefore, that the pressure in a leg vein with the limb at rest should equal the pressure exerted by a column of blood extending vertically from the vein observed to the level of the heart

That this is so has been shown by Smirk (8) in the course of investigations on cardiac œdema, by Beecher (4) while investigating the flow of tissue fluids in the limbs, and by Pollack and Wood (7) who were investigating normal venous physiology Our own results, which were obtained from all the patients investigated, whether their veins were normal or abnormal are

FIGURE I
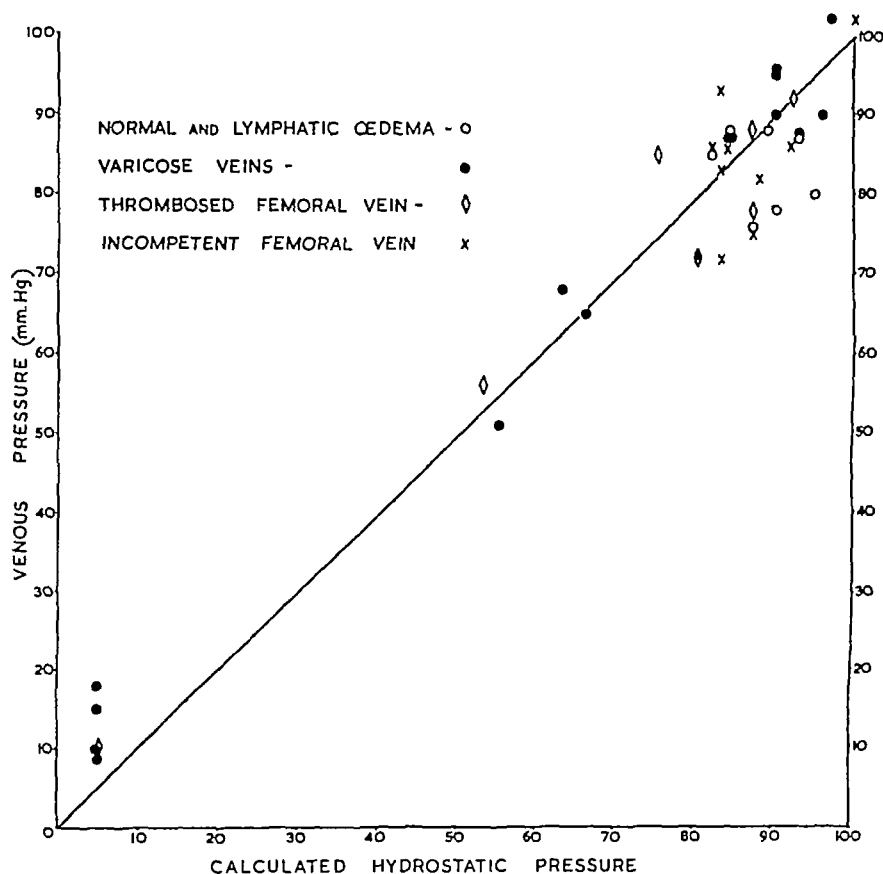
Fig 1 In this graph the venous pressures measured in the foot with the patients still (i e , not exercising) are plotted against the expected hydrostatic pressures calculated from the vertical height of the xiphisternum above the level of the foot The line drawn is that of exact correspondence between the two The points fall fairly close to it The figures are given in Table I, columns 1 and 2

given in Table I and Fig 1 Here the venous pressure in the foot* is compared with the calculated pressure exerted by a vertical column of water

* All pressure readings in this paper are expressed as millimetres of mercury

extending from the foot to the xiphisternum   It will be seen that the venous pressure in the foot at rest is related directly to the vertical height of the xiphisternum above it, and that this is so whatever the state of the leg veins   The pressure in a vein of the foot in a man of ordinary height when standing still is of the order of 90 mm Hg

During exercise, in the normal person, a second well known factor comes into play, namely the compressing action of the muscles acting on patent yielding veins with functioning valves   The blood is thereby lifted from the foot towards the heart and the pressure in the veins of the foot is so diminished that the superficial veins may sometimes be seen to empty   In normal people, the pressure in the foot veins during walking has been shown by the foregoing observers to fall markedly, to a level of about 20-30 mm Hg , and we have assumed that the extent of the fall would furnish a measure of the overall efficiency of the leg veins

A technique of venous manometry

The problem of measuring venous pressures during exercise has been solved by the introduction of translucent, flexible, non-irritant polythene tubing   Direct cannulation was preferred to the use of a capsule as described by Beecher since direct are always preferable to indirect measurements, and since in the presence of much œdema or thickening of the subcutaneous tissues, a vein suitable for a capsule might not be available   The apparatus used consists of an ordinary mercury sphygmomanometer connected by way of a three way cock to its air pump and to a removable sterilizable bottle   The air pressure in this bottle can therefore be regulated and measured   The bottle is half filled with heparinized normal saline, and a glass tube, narrow at its outer end, passes through the cork and reaches almost to the bottom   Into this tube is inserted one end of a 5 foot length of medium sized (Size 2) polythene tubing (Allen and Hanburys), an air tight joint between the two being made by a piece of narrow rubber tubing

The other end of the polythene tube is inserted well into a vein on the dorsum of the foot in the conscious patient by cutting down on the vein under local anæsthesia   The tube is tied in, the skin is closed by two fine stitches and a firm elastoplast dressing is applied, the tip of the tube in the vein being clear of the elastoplast   The patient, who should have had no pre-operative medication and who should understand what is required of him, then stands perfectly still   The manometer is put at the same level as the patient's foot and blood will be seen to rush into the translucent polythene tube   It is checked by raising the pressure in the bottle to about 90 mm Hg   This pressure is then adjusted until the blood-saline junction remains stationary   The manometer reading is recorded and repeated several times   The height of the sterno-xiphoid joint is measured to allow comparison between the actual and expected pressures

TABLE I

This table sets out for each patient the calculated pressure exerted by a column of water extending vertically from the foot to the level of the xiphisternum, the venous pressure measured in the foot at rest (the patient standing erect unless otherwise stated) and the venous pressure measured in the foot during exercise with and without a tourniquet below the knee. In the "incompetent femoral vein" group are added the resting and exercise pressures after ligation of the popliteal vein, measured without tourniquet. All pressures are in mm Hg

	Calculated hydrostatic pressure	Pressures at rest		Pressures during exercise		
		Without tourniquet	After ligation of popliteal vein	Without tourniquet	With tourniquet	After ligation of popliteal vein
Normals						
A A H	95	80	—	17	—	—
P S	84	88	—	28	32	—
D P	93	87	—	0	—	—
E L	90	78	—	0	—	—
Lymphatic œdema						
A H	80	88	—	33	—	—
Q W	82	85	—	30	—	—
I C	87	70	—	18	—	—
Varicose veins						
B	90	90	—	—	—	—
B (flat)	5	9	—	—	—	—
H L (inclined)	97	102	—	59	25	—
H L (flat)	55	61	—	—	—	—
L C	5	10	—	—	—	—
L C (flat)	87	84	—	64	35	—
M C	87	84	—	64	6	—
M C (flat)	5	15	—	—	—	—
A.B (inclined)	95	90	—	35	34	—
A.B (flat)	65	66	—	—	—	—
B R	90	90	—	20	15	—
B R (inclined)	63	68	—	—	—	—
B R (flat)	5	18	—	—	—	—
B J R	90	90	—	58	15	—
P I	93	88	—	30	15	—

TABLE I—*continued*

	Calculated hydrostatic pressure	Pressures at rest		Pressures during exercise		
		Without tourniquet	After ligation of popliteal vein	Without tourniquet	With tourniquet	After ligation of popliteal vein
Thrombosed femoral vein						
W J (inclined)	87	88	—	00	58	—
W J (inclined)	53	50	—	—	—	—
E P (inclined)	87	78	—	02	02	—
E P (flat)	05	10	—	—	119	—
J O	93	92	—	100	—	—
D.R (left leg)	80	72	—	00	—	—
D R (right leg)	80	72	—	54	—	—
Incompetent femoral vein						
M.A	82	86	70	53	63	35
B	88	82	70	56	56	19
G B	84	94	04	100	—	80
J L	92	80	—	05	—	—
*R.M	100	102	105	108	108	74
†*A G H (left leg)	84	80	85	90	80	80
†A G H (right leg)	84	84	83	80	80	01
†*M H	84	72	70	55	83	04
A B H	97	75	—	80	75	—

* Previous ligation of the superficial femoral vein.
† Previous lumbar ganglionectomy

　The patient is then asked to mark time smartly, raising each foot 9″ sixty times per minute and readings are repeated as above　Several consistently close readings are usually obtained after the first few steps

　The exercise reading is repeated with a rubber tourniquet in place below the knee in an attempt to cut off the flow in the superficial veins at this level　This is a handicap to venous return if the deep system is blocked at this level but improves the return if the superficial system is incompetent, and these alterations are reflected in the exercise pressure

　The tube is then clamped close to the foot and cut off here, an ascending phlebogram* being made, with the patient horizontal and the superficial veins obstructed at the ankle, using the polythene tube as a cannula for the injection, a 15″ × 12″ cassette is used behind the upper leg　This outlines any blocks in the greater part of the femoral and popliteal veins　At the conclusion, the polythene cannula is withdrawn without disturbing the dressing and a pressure bandage is applied over the elastoplast

　This technique provides three pieces of information　the venous pressure in the foot with the patient standing still, the pressure on exercise, and a record of the anatomical state of the popliteal and most of the femoral vein

　The method has a satisfactory degree of accuracy for clinical work For pressure measurements made with the patient standing still, consecutive readings are very close, and a scatter of more than 4 mm Hg is exceptional Readings at rest obtained in the same patient at intervals of several days, a different vein in the same foot often having been employed on the two occasions, gave mean figures varying by 0-7 mm Hg, average 3 5 in 7 patients

　The exercise readings are a little more difficult to get because of the movement of the polythene tube and because of variations in the patient's effort　Pollack and Wood's (7) careful work on the normal has shown that there is little change in pressure whatever the speed of walking over 1 7 m p h　Thus usually, provided a moderate effort equivalent to a slow walk is maintained or exceeded, the venous pressure is kept about constant at its exercise level　While it is not possible by the simple method of marking time which we have used to ensure that all patients undertake precisely similar degrees of effort, the effort prescribed is considerably in excess of that required to maintain the pressure at its exercise level, so that comparable exercise pressures are obtained　The scatter of readings is usually within 10 mm Hg

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　* By " ascending phlebography " is meant the injection of radio-opaque medium into a vein in the distal part of the limb, X ray films are then made of more proximal parts of the limb and demonstrate the filling of the veins by the medium moving with the venous current in the normal (centripetal) direction

　By " descending phlebography " is meant the injection of radio opaque medium into a main vein at the root of the limb, the films are then made of the more distal parts which are angled to 45° below the site of the injection　Gravity will cause some of the medium to run in a centrifugal direction

In some abnormal cases however, we have found that variations of effort about the prescribed level produce fluctuations of as much as 20 mm Hg   This in itself is an abnormal finding   In interpreting the results, the scatter of the readings must be borne in mind but no difficulty arises in practice

If the available vein will admit only the small size of polythene tubing (Size 1 A   and H) the increased friction renders the exercise pressures a little more difficult to obtain and the scatter is wider   No inconvenience has followed the observations other than mild inflammation around an unabsorbable linen ligature   The procedure can be readily carried out on out-patients

Findings in untreated cases

Normal leg veins   4 patients were volunteers having nothing to suggest any abnormality of the leg veins tested   One was a man who had an unexplained pain in the legs and one had a mild coarctation of the aorta and a diminished flow of blood into the legs   They were active men, aged 20-32

The pressures measured are given in Table I and the exercise falls are shown in Fig 2   It will be seen that the exercise pressures vary between 0 and 28 mm Hg

The figures obtained by other investigators who measured venous pressures during exercise near the ankle in normal subjects are summarized in Table II   Smirk's (8) technique did not allow the foot to be raised and he was therefore unable to imitate walking , there was a large scatter in his exercise readings, which are the first to have been reported   Beecher's (4) results with " high steps," the heel being raised 25 cms from the ground 40 times a minute, and Pollack and Wood's (7) with actual walking in a treadmill appear to be comparable with ours

Excluding Smirk's (8) figures, therefore, it would seem that the highest walking pressure which can be considered as compatible with a normal deep venous system is probably 40 mm Hg

Lymphatic œdema   Three patients have been investigated, all women, who had had œdema of one or both legs for 35, 8 and 1 years   None had symptoms of venous disease   Their only trouble was a heavy aching feeling in the ankle due to the weight of the œdematous tissue   Ascending phlebograms were normal in each case

The pressures measured are given in Table I and Fig 2   Since all were below 40 mm Hg   these results were thought to be good evidence that the œdema was of lymphatic and not venous origin

Superficial varicose veins   The 7 patients investigated all had marked reflux in the great saphenous system   Two had associated ulcers and one had varicose eczema   Apart from discomfort around ulcers or distended

veins, pain was absent in this group　The veins on the dorsum of the foot emptied readily with exercise when a tourniquet was applied below the knee in all cases　In 5 of the cases œdema did not occur

Two of the patients with skin changes had a history of slight intermittent œdema of the ankle　Both of these are of particular interest　The first (B R) exhibited varicose eczema without ulceration　The ascending phlebogram showed normal filling　Despite the easily demonstrable saphenous reflux, the exercise pressure even without a tourniquet fell to 20 mm Hg　Clearly the venous pump may have a large working margin

FIGURE 2

EFFECT OF EXERCISE ON VENOUS PRESSURE IN FOOT
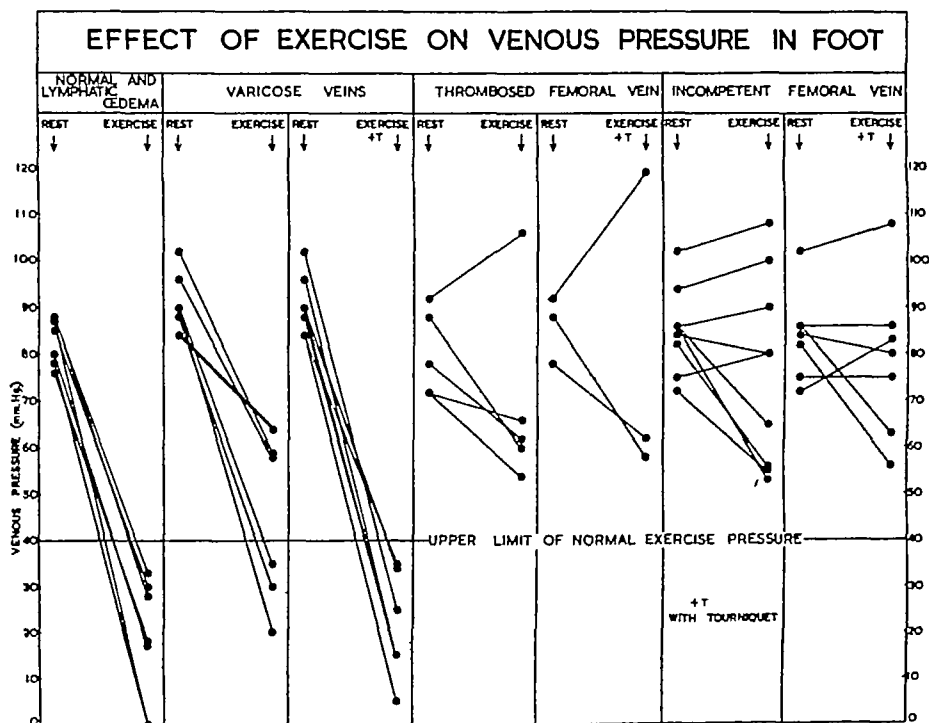
Fig 2　Shows the effect of exercise on the venous pressure in the foot in subjects with normal and diseased veins　Each line shows the change in pressure for one subject resulting from exercise, with or without a tourniquet below the knee

since it can empty the leg satisfactorily, as shown by the normal exercise pressure, though handicapped by a considerable leak back through the superficial vein　The second (P I) exhibited ulceration　He gave a history of white leg 6 years before following pneumonia　Ascending and descending phlebograms showed patent deep veins without reflux, and on exercise with a tourniquet the venous pressure fell to 15 mm Hg　The thrombus which must have been present in his deep main veins had evidently been removed with preservation of functioning valves, a remarkable event considering the delicacy of their structure

In general, œdema was not a feature of this group and the precise reason for its occurrence in two cases, though to a minimal extent, is not apparent No abnormality of the deep veins was demonstrated in this group

The pressures recorded are tabulated in Table I and shown graphically in Fig 2 It will be seen that the exercise pressures remain much higher in most cases than in the groups with normal veins This is another expression of the fact, well demonstrated by watching the superficial foot veins in exercise, that gross reflux in the superficial system usually impedes emptying of the foot veins by a normal deep system The considerable handicap to venous return which these figures clearly demonstrate, however, may not produce skin changes and commonly does not produce œdema The use of a tourniquet to obstruct the superficial veins below the knee reduced the exercise pressure to about normal levels In three cases in which the exercise pressures were again read after completing the operation for saphenous reflux, the readings were intermediate between those obtained with and without tourniquet beforehand That is to say the reflux was less well controlled by the operations than by the tourniquet (Table III) The operation consisted of ligation of the great saphenous vein at its junction with the femoral vein and of all its tributaries at this level , the great saphenous vein was also ligated in the lower third of the thigh and in some cases in the upper third of the calf as well

Thrombosed femoral vein The 4 patients in this group had swelling of the legs and recurrent ulceration or dermatitis about the ankle, dating from trauma or an infection of the leg between 1½ and 10 years previously In one case there was a heavy feeling in the limb , otherwise, apart from the ulceration, pain was absent Ascending phlebograms and descending phlebograms were done in each case, and showed a block of the lower part of the femoral vein The results are shown in Table I and Fig 2

It will be seen that the exercise pressures are high and differ from the varicose vein group in remaining high, when a tourniquet is applied below the knee to compress the superficial veins

Incompetent femoral vein In this group of 8 patients, one of whom had both legs affected, pain of a bursting character in the dependent leg was a constant feature Swelling of the leg was also constant , ulceration or dermatitis occurred in 4 In 5 cases there was a clear history of antecedent thrombo-phlebitis following trauma, childbirth, or pneumonia In the remaining cases the onset was less clear cut and may have been due to a quiet phlebothrombosis, or to a primary valvular failure akin to that occuring in varicose superficial veins Descending phlebograms were done in all limbs but one and showed patent incompetent femoral veins, the contrast medium in the femoral vein running readily downward toward the knee from the groin

On the evidence of the descending phlebogram three of the patients had been treated by ligation of the superficial femoral vein in the groin before pressure measurements in the foot were undertaken   Ascending phlebograms were done in all limbs but one (in which the descending phlebogram showed a patent incompetent femoral vein) at the time of the pressure measurements   In the untreated patients patent femoral veins were outlined   In those in whom femoral ligation had been done the contrast medium readily found its way upwards past the ligature by large channels such as the profunda femoris vein   This fact and the pressure measurements suggested that the ligatures had not materially altered the venous function , and these limbs are therefore considered with the untreated cases of incompetent femoral veins   The exercise pressure are shown in Table I and Fig 2

It will be seen that the exercise pressures are high and are not decreased by the use of a tourniquet which compresses the superficial veins   They thus do not differ from the exercise pressures found in the femoral vein block group   These two groups are differentiated by the phlebogram which shows a patent femoral vein in the incompetent group   An ascending phlebogram is clearly sufficient for this purpose, so that an incision in the groin and descending phlebography are unnecessary according to our observations   In the group presented here descending phlebography was necessary in order to select cases of incompetence on which pressure observations could be made   It is, of course, a valuable confirmatory step

Findings after ligation of deep veins

Ligation of the superficial femoral vein   (Table I)   Three patients treated by this method are referred to in the previous section   No change in their clinical condition was noted, a disappointing result   In one patient having both legs similarly affected femoral ligation had been performed on one side   A year later no difference whatsoever could be made out between the two limbs clinically or radiographically except that phlebograms showed the contrast medium to be taking different courses in the two limbs   The exercise pressures in the two limbs were the same

Ligation of the popliteal vein   Ligature of the popliteal vein is a procedure of promise and was introduced by Gunnar Bauer (3) in an interesting paper in 1948   In it he divides his cases of femoral venous incompetence into two etiological groups, those due to recanalization of a previously thrombosed femoral vein and those arising as a primary mechanical valvular failure   The presenting symptoms are a bursting feeling in the calf when it is dependent, usually associated with mild œdema and cyanosis of the foot   These symptoms are relieved by elevation of the limb   Malleolar ulceration difficult to heal, often associated with pigmentation and subcutaneous fibrosis and eczema of the lower leg, usually develops in time

Selecting cases by these criteria he establishes the diagnosis by means of a retrograde phlebogram, which distinguishes them from those having a blocked vein   This procedure has been commented on above   Six of the patients (7 limbs) in our incompetent femoral vein group have been investigated and treated by his methods   Ascending phlebography and venous pressure measurements were also done   Of these, three had had previous superficial femoral vein ligation performed without benefit as mentioned above

TABLE II

This table summarizes the findings of previous authors on normal subjects   The figures have been converted into mm Hg

Authors	No of subjects investigated	Type of exercise	Exercise pressures recorded		Method
			Range	Average	
Smirk	5	Flexing knees and ankles every ¼ sec	67–25	Insuff data	Venepuncture and water manometer
Beecher	19	40 steps per min. Low steps High steps	35–11 25–7	21 15	Indirect method using capsule to collapse veins
Pollack and Wood	10	Walking at 1 1 m p h 2 6 3 3	25 3–11 38 –13 43 2–10	22 3 24 3 23 6	Venepuncture measurement with strain gauge mano meter

TABLE III

Effect on venous pressure of operation for varicose veins

	Pressure at rest-standing	Pressure during exercise pre-operatively		Pressure during exercise post-operatively without tourniquet
		Without tourniquet	With tourniquet	
H L	100–3	59	25	37
L C	84	64	35	57
M C	84	64	5	40

The operation of popliteal vein ligation was performed according to the method of Gunnar Bauer (3) through a vertical 3″ incision in the upper half of the popliteal space   The vein was tied and divided and a piece taken for section   Several such segments showed evidence of past thrombosis   The venous pressure measurements were repeated 10 to 14 days later with the patient fully ambulant   The results as reflected by the exercise pressures are shown in Table I

Four of the 7 treated limbs show a considerable improvement in the exercise pressures and this was associated with marked amelioration of pain Of the improved limbs one had previously been treated by superficial femoral ligation , none had had a lumbar sympathectomy Of the three limbs showing no response clinically or by pressure measurements, two had had superficial femoral ligation All three had had a lumbar sympathectomy performed previously and the venous dilatation associated with this may possibly have been a factor in preventing response These results in so intractable a condition are distinctly encouraging It will be seen that the best results were obtained in the two cases with the lowest exercise pressure before operation

COMMENT

The technique of manometry described is easy to carry out, and provided that the patient is co-operative and that size 2 polythene tubing can be used, which has been so in nearly all our cases, gives reliable readings It is not suitable for children who are apt to be too nervous, but, apart from arterio-venous malformations, venous disease is rare in them Its value lies in assessing the efficiency of the venous return in the leg in exercise, particularly in the deep venous system, which is mainly responsible, when acted upon by the skeletal muscles, for returning the blood It seems as far as our experience has gone to be a most useful investigation when dealing with such symptoms as pain, swelling, or ulceration in the leg which may be the result of venous disease, and particularly is this so when treatment on the lines of deep venous ligation is contemplated

Some points of interest emerge from our findings The discrepancies in the readings obtained in cases with saphenous reflux when taken with a tourniquet in place and again after apparently adequate operation have been mentioned The explanation that suggests itself is that other communicating channels not interrupted at operation exist between super-ficial and deep systems, allowing reflux into the former and were undetected before operation Though the evidence is somewhat indirect, in that no exercise pressure readings were done before operation, it seems that ligation of the superficial femoral vein in cases of femoral incompetence produced no observable changes in the venous function This is in striking contrast to the effect of popliteal vein ligation which in some patients produced a very clear improvement

There are two reasons why ligature of the main venous channel with incompetent valves may improve the venous return In the first place the blood may be diverted to collateral channels having competent valves If, however, these vessels dilate and hypertrophy in response to the added load their valves may soon cease to be competent

In the second place if the valves in all the veins in the limb at the level of the ligature are incompetent, the effect of the ligature will only be to

diminish the total capacity of the veins at that level   If the collaterals together have a relatively small cross section a resistance to the venous return will be created   It is suggested as a working hypothesis that the effect of such a resistance may be as follows   As a result of the muscular contraction of walking blood is expelled from the muscle veins against the capillary resistance on the one hand, and against the resistance of the venous outlet from the segment of the limb under consideration on the other   The capillary resistance is high, since, in the lower leg at any rate, it is sufficient to support venous blood at a high pressure while standing still   Hence venous blood will be forced out through the narrowed venous outlet at a pressure depending on that developing within the contracting muscles   That this pressure is sufficient to expel venous blood from the calf against a resistance of 90-100 mm Hg has been shown by Barcroft (1)   he also showed that the pressure in the calf muscles in certain circumstances may exceed the arterial pressure (2)   During the phase of relaxation of muscle the pressure gradient about the level of the ligature will be reversed, a low pressure developing below the level of the ligature and the static pressure of the column of blood reaching from there to the heart (in a totally incompetent system) persisting above it   Hence venous blood will sink back below the ligature level   If the pressure developed during contraction is sufficiently high and is maintained for a sufficient proportion of the contraction-relaxation cycle, then more blood will be forced past the resistance of the venous outlet from below than returns past it from above, and the net effect of the ligature will be to improve the upward flow of blood   It is difficult to know how great a part competent collaterals play   Retrograde phlebograms in cases of valvular incompetence sometimes show reflux in what seem to be all the veins of the thigh suggesting that few if any competent collaterals exist   The explanation of the difference in the results of superficial femoral and popliteal ligation in this series is probably to be found in the presence of many incompetent collaterals in the thigh

Popliteal ligation, in contrast with superficial femoral ligation, leaves very few collaterals, which enhances the chance of success if the foregoing arguments are accepted, and also protects the lower leg to some extent from the additional hydrostatic pressure developed in the thigh   It is clearly a more efficient procedure than superficial femoral ligation   Lumbar sympathectomy seems to be a harmful procedure in these cases   Not only does it tend to increase the input of blood into a limb which already has difficulty in emptying itself, but also causes dilatation of the veins which jeopardizes valvular competence   In a few patients in each group, exercise pressures have been measured with an elastic bandage covering the lower limb   These pressures were found to be the same as those obtained in the same patients with a tourniquet below the knee   (Table I)   So it appears that the relief experienced by patients when wearing elastic support on the leg is due to an improvement in the venous return only in cases of incompetent

superficial veins , in deep venous disease no improvement in the exercise pressure when wearing an elastic bandage was found   Even so, the control of œdema by the supporting effect of such bandages is a valuable measure

Summary

1   A simple technique of measuring the pressure in the veins of the foot at rest or during exercise is described   It has been applied to the problem of venous lesions of the legs, particularly with regard to measuring the efficiency of the venous return from the limb and to assessing the results of venous ligation   In these problems it has proved very useful

2   The venous pressure in the foot when standing still, whatever the state of the main veins of the limb, approximates to the pressure exerted by a column of blood extending vertically from the foot to the heart   This pressure is of the order of 90 mm Hg in a man of ordinary height

3   The venous pressure in the foot during exercise (marking time), when the main veins of the limb are normal, falls to under 40 mm Hg , such a fall indicating high efficiency of the musculo-venous pump and a normal venous return

4   The venous return may be impaired in varicose veins due to valvular incompetence of the great saphenous vein and is considerably improved by ligation of the incompetent veins

5   The venous return is markedly impaired by thrombosis or valvular incompetence of the femoral vein   In some cases of the latter type ligation of the popliteal vein will greatly improve the efficiency of the venous return as judged by notable reduction of the exercise pressure   It is suggested that lumbar sympathectomy and consequent venous dilatation may interfere with the success of this procedure   Ligation of the superficial femoral vein in cases of femoral valvular incompetence could not be shown to improve the venous function

6   The application of elastic bandages to the leg improved the exercise pressure only in cases of superficial varicose veins

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THE COMPARATIVE VALUES OF DEXTROAMPHETAMINE SULPHATE, DRIED THYROID GLAND AND A PLACEBO IN THE TREATMENT OF OBESITY

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PREPARATIONS containing thyroxine have been commonly used in the treatment of obesity for the past fifty years   The rationale claimed for their use has been that they stimulated metabolism, raising the basal metabolic rate either to a normal level in patients who were presumed to have a low B M R   or to a level above the normal in those with a normal rate   More recently drugs have been sought which might decrease the appetite or relieve hunger without altering the overall level of metabolism , amphetamine sulphate (Benzedrine) and later the dextro-rotatory isomer (Dexedrine) have been used for this purpose and have gained a wide popularity in the treatment of obesity   No claim has been made that amphetamine increased the metabolic rate or the activity of the subject but its action has generally been considered to be a central inhibition of appetite (3)

None of the published reports on the use of thyroid and dextro-amphetamine sulphate in the treatment of obesity appear to have been based upon a fully controlled experiment   In the following paper the effects of thyroid, dextroamphetamine sulphate and placebos upon the weight loss of obese subjects on a 1000 Calorie diet are compared, using an experimental design which eliminates the major sources of random error

Method

Patients referred to the Hospital Endocrine Clinic for obesity, endocrine disorders with obesity, or subfertility with obesity were the chief source of material, together with a few patients presenting themselves at the medical outpatient department with complaints not necessarily related to their obesity   Obesity was roughly defined as an excess weight of more than 30 lbs over the height-weight relationship given in Table I in the case of women , and for the two children as of more than 20 lbs over the height-

weight-age data given by Duncan (2)   No men were included in the series
The variation of body weight with age given in the standard height-weight-
age tables published by the Association of Life Insurance Directors and
Actuarial Society of America (1) was not considered to be relevant to the
definition of obesity and no age allowance was used   No distinction was
made between patients who presented themselves with the chief complaint
of obesity and those in whom it was considered a wise therapeutic measure
to decrease their weight   Unless the patient wanted to lose weight, with or

TABLE I

Ideal height weight relationship for mature female subjects of all ages (unclothed)

Height	Weight	Height	Weight
inches	lbs	inches	lbs
55	102	64	125
56	104	65	128
57	106	66	132
58	108	67	136
59	110	68	140
60	113	69	144
61	116	70	148
62	119	71	152
63	122	72	156

without persuasion, she was not included in the series   Patients with
endocrine disorders were excluded, as were those suffering from ailments
which might be influenced unfavourably by the administration of dextro-
amphetamine sulphate or dried thyroid B P   Because a rapid loss of
weight was expected at the initial stage of reduction, only those patients
who had not lost any weight for some months were included in the trial

All subjects were treated as outpatients and given the same standard
1000 Calorie diet (Table II) which was explained to them by a dietitian
The importance of adhering strictly to the prescribed diet and its role in
reducing their weight was impressed upon each patient at their first and at
most of their subsequent visits by one or both of the authors and it was
stressed that the Calorie deficit and not the box of tablets caused the loss
in weight   The tablets were described as being designed to reduce the
patients' appetite and improve their sense of well being, thus aiding them to
discipline themselves and manage with less than their customary intake of
food   They were also assured that when they had lost enough weight they
would not have to continue on the 1000 Calorie diet but would be given
guidance on the use of a normal diet suitable for their requirements, the
reducing diet being designed to re-educate them to better eating habits as

TABLE II

Reducing diet 1,000 Calories

	Approx Amount	
Breakfast	2½ oz	Milk for tea or coffee, no sugar
	1 oz	Bread
	¼ oz	Butter or margarine
	1	Egg, boiled or poached, or alternative
	4 oz	Fruit or tomatoes (an average helping), when available
Mid morning		Marmite, oxo or bovril to drink, or tea or coffee with milk from day's ration, no sugar
Dinner	2 oz	Lean beef or alternative
		Green vegetables (a good helping)
	5 oz	Root vegetables (a good helping), see alternatives
	4 oz	Fruit (an average helping), see alternatives
Tea	2½ oz	Milk for tea, no sugar
	1 oz	Bread
	¼ oz	Butter or margarine
		A good helping of salad and/or fish or meat paste or marmite
Supper	4 oz	White fish or alternative
		Green vegetables or salad (a good helping)
	1 oz.	Bread
	¼ oz	Butter or margarine
	4 oz	Fruit
Before retiring	5 oz.	Milk

SUMMARY OF DIET		CHO gm	P gm	F gm
½ pint	Milk	14	9	11
3 oz	Bread	47	7	1
¾ oz	Butter or margarine	—	—	18
16 oz.	Fruit	25	—	—
2 oz.	Lean meat	—	14	4
4 oz	White fish	—	20	4
1	Egg	—	7	7
5 oz.	Root vegetables	5	—	—
		91	57	45

BREAKFAST ALTERNATIVES

Instead of 1 egg take any of the following foods in the quantities stated —

1 oz	Lean bacon, grilled
2 oz	Kidney
3 oz.	Kipper baked with bone
2 oz.	Herrings, baked in vinegar
3 oz	Haddock, steamed with bone, plus one-eighth oz margarine
2 oz.	Baked beans, omit fruit when this alternative is used or
1 oz.	Bemax plus milk from day's ration. Substitute 1 plain biscuit for the bread and use a scraping of butter instead of ¼ oz Omit fruit.

TABLE II—*(continued)*

DINNER ALTERNATIVES

Instead of 2 oz lean beef take any of the following —

2 oz	Lean mutton, veal or ham
2 oz	Corned beef, liver, rabbit or chicken.
2 oz	Tinned salmon, pilchards or sardines, drained of oil

Root vegetables allowed are Carrots, onions, swedes, turnips, leeks

Fruits allowed are Apples, pears, oranges, plums, fresh peaches, fresh raspberries, blackberries, strawberries, tangerines, damsons, cherries, blackcurrants

Melon, rhubarb, gooseberries, loganberries, grapefruit may be taken in slightly larger quantities Bananas, grapes and dried fruits such as prunes, apricots and figs should be taken in very small amounts

SUPPER ALTERNATIVES

Instead of 4 oz white fish take any of the following

4 oz	Tripe	1 oz	Cheese
4 oz	Sweetbread	3 oz	Soused herrings
4 oz	Heart	1¼ oz	Liver
1¼ oz	Meat		

IMPORTANT

1 Do not take any of the following foods —Sweets, chocolate, sugar, potatoes, cakes, pastry, puddings, jam, marmalade, honey, syrup, fruits preserved in syrup, fried foods, sausages, thickened soups and sauces, alcohol

2 Take only the amount prescribed of bread, biscuits, milk.

3 Salad and green or root vegetables may be taken in moderately large amounts when other foods are lacking

4 Fruits, if cooked, should be sweetened with saccharin , add the saccharin just as cooking is completed to avoid bitter taste

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well as to reduce their weight It was suggested that during the first month they might expect to lose about 10 lbs and during the succeeding months about 6 lbs per month Each patient attended at 4-weekly intervals , if this interval was changed the treatment was continued but the patient was excluded from the series

At the first visit the patient was given a general medical examination and the height in bare feet and weight in underclothing was recorded At subsequent visits the weight in underclothing was recorded and also the height in the case of those who were not yet fully grown This was followed by discussion and record of the ability to follow the diet, the effect of the tablets given and general health and intercurrent illness At each visit the patient was given a box of tablets labelled A, B, C or D

Each box contained 84 brown and 84 yellow tablets The brown tablets contained either 30 mg of dried thyroid B P or a placebo of peptone and burnt sugar Some difficulty was experienced in preparing a placebo identical in appearance with the thyroid-containing tablets and some batches of the placebo could be distinguished from the thyroid preparation This difference was remarked upon by one or two patients who were then

told that it was merely a difference in the preparation batch The active yellow tablets contained 5 mg of dextroamphetamine sulphate and could not be distinguished from the inactive placebos The identity of the preparations was known only to the pharmacist who prepared them until the end of the experiment The tablets were dispensed in the following combinations A = brown placebo and yellow placebo , B = brown placebo and yellow dextroamphetamine sulphate , C = brown thyroid and yellow placebo , D = brown thyroid and yellow dextroamphetamine sulphate The patient was instructed to take one brown and one yellow tablet on rising, half an hour before the midday meal and in the middle of the afternoon Where this was impracticable because of working or feeding conditions a reasonable modification was arranged Dextroamphetamine sulphate was not given later in the day than 5 p m so as to avoid possible insomnia

Possible sources of error and variation in the rate of weight loss

In comparing the weight lost month by month in a given patient or that lost in different patients, errors may arise from many sources Some which are relevant to the design and interpretation of the experiment are discussed below

(*a*) *The Calorie intake or the ability of the patient to follow the diet* A uniform daily intake of 1000 Calories was assumed in each patient although wide variation must have occurred Intake is greatly influenced by the intelligence of the patient and the dietitian , the time and thought which the patient gives to the measurement of her diet , the desire of the patient to lose weight and adhere rigidly to the regime , and her hunger, whether it be physiological or psychological

As well as such individual variation in Calorie intake, variation between successive months may arise in several ways During the first month the patient may limit her intake only moderately in the fear that she must eat " to keep her strength up " At the next visit, reassurance and encouragement to lose more weight by eating less food may result in a change in attitude and Calorie intake The loss of 10 or more pounds during the first month may act as a considerable encouragement to the patient to continue, but it may have the reverse effect , such a loss may, in spite of reassurance be considered harmful, or unnecessarily rapid and used as an excuse to slacken the discipline , or as time goes on the patient may tire of the regime or become less careful with the diet The character of the patient and her faith in the physician and his treatment are all-important factors in prognosis

(*b*) *The initial weight and surface area* These factors introduce a variable between one patient and another and a variation with time in a given patient The weight of the patient affects the Calorie expenditure in two ways, since the greater the bulk the greater the energy required to maintain the basal metabolism and since variations in bulk are associated with variations in activity

(c) *The Calorie deficit*   The weight loss in unit time is directly related to the Calorie deficit, or the difference between energy intake and energy expenditure   This introduces wide variations in rates of weight loss from patient to patient since some patients, because of their occupation or their attitude to life, expend much more energy than others regardless of their weight

(d) *The calorific value of the tissue lost*   The type of tissue consumed during the process of weight loss introduces a variable in the rate of weight loss with time   During the initial stages of reduction fat is not the only tissue utilized , carbohydrate stores are depleted and there is evidence that protoplasm is consumed (4)   The energy released per gram of these tissues is less than that released per gram of adipose tissue, so that on a constant Calorie deficit the rate of weight loss is greater during the first week or perhaps two weeks than subsequently when the chief if not only source of stored energy is adipose tissue

(e) *Unknown factors*   The metabolic changes associated with a Calorie deficient diet when there are stores of energy in the form of fat are not completely known   Apart from changes in protein and carbohydrate utilization there may be a complex change in metabolism which manifests itself as a change in the basal energy consumption   Unknown factors of this nature have been assumed, without definite evidence, to act similarly in all patients

(f) *Water retention and contents of viscera*   Temporary variations in the fluid content of the tissues occur during weight loss   Frequently a patient who is on a constant Calorie deficit fails to lose weight for several days, and may even gain weight   Determinations of the water balance show that these fluctuations from the expected weight loss curve are due to retention of water and when diuresis occurs the weight returns to the predicted level   Many women, regardless of the Calorie deficit, store water premenstrually and have a diuresis about the time of onset of the period   The quantity of water stored may amount to several pounds in weight and result in a serious error

Variations in the contents of the stomach, bowel and bladder may amount to several pounds in weight, but it was not practicable to ensure that bowel and bladder were emptied just prior to weighing or that a constant weight of food and fluid had been consumed at a constant time interval before weighing

(g) *Seasonal variations*   Holidays in boarding houses, staying with friends, or warm and cold weather introduced variations in the desire of the patient to eat   The availability of such articles of food as salads, fruit, green vegetables and tomatoes and fluctuations in their prices made the ease of maintaining a constant diet dependent on the time of the year

(*h*) *Intercurrent illness*   Where illness meant a complete change of activity and food intake for more than a few days in the month the patient was omitted from the series, but many patients had colds or minor gastro-intestinal disorders which affected their ability or desire to maintain their diet for short periods

The control of variables and the plan of the experiment

Errors caused by variations from patient to patient and from month to month were reduced by using a complete randomised block subdivided into a group of Latin squares   Four treatments were employed   A, using placebos only, B, using dextroamphetamine sulphate only, C, using thyroid only, D, using both dextroamphetamine sulphate and thyroid   Each patient received each treatment for one month and all possible sequences of treatments were used   This involved 24 patients each being treated for 4 consecutive months   The first administration of any one treatment was in random order and patients were allotted to treatment sequences as they were seen in the Outpatient Departments   When a patient was discarded from the series, her place was filled by the next new patient seen   The orders of treatments and patients, together with other data, are given in Table III   An analysis of variance was made on this material

Two hundred and fifty observations were made on patients who were rejected at some stage or who, after completing the four month period, continued to be given the treatments A, B, C, D in random order   These data were also treated by analysis of variance after making suitable corrections for their biased distribution   The data for this analysis were not so reliable as those for the complete randomised block

It will be clear that, since patients were rejected if they failed to complete the four months' treatment, the results on the randomised block apply selectively to those patients with sufficient faith, determination or suscepti-bility to regimentation to adhere to the regime

Results

A summary of the analyses of variance on the two sets of data is given in Tables IV and V   The important points in the results are as follows —

(1) Dextroamphetamine sulphate (5 mg   three times a day) had a highly significant effect in increasing weight loss when used in conjunction with a 1000 Calorie diet

(2) Thyroid (30 mg   three times a day) had no significant effect in similar circumstances

(3) Dextroamphetamine sulphate and thyroid neither augmented nor diminished the effects of each other

(4) The effect of dextroamphetamine sulphate persisted over the four month period of treatment and there was no significant difference between its effects in different months

TABLE III

Experimental data

No. of patient	Order of treatment	Initial weight lbs	Weight at end of each month in lbs				Total weight lost lbs	Height ins	Age yrs	Original excess weight lbs
			1st	2nd	3rd	4th				
2b	DCAB	110.5	100.5	98	95.5	92	18.5	52	9.5	40.5
3	ABCD	132	124	118	117	115.5	16.5	60.5	28	18
28	DABC	156	144	145	141	142.5	13.5	63.5	24	33
20	BACD	190	171	166	159	153	37	63.5	42	67
54	CBAD	180	172.5	162	158	147	33	58.5	51	71
7	BCDA	133	119	114	108	106	27	62.5	12	30
8	DCBA	247	231	221	212.5	205	42	64.5	38	120
33a	CDAB	214	200	185.5	183	178	36	65	27	85
10	ADBC	152	142	133	128.5	126.5	25.5	62	25	33
34	ABDC	174	161	151	147	143	31	65	34	45
11a	ACBD	182	173	157.5	150	142	40	63	38	00
59	CDBA	233	212.5	197	184	176	57	64.5	18	100
12a	BDCA	139	133	127.5	123.5	121	18	55.5	41	30
37	BDAC	209	200	192.5	190.5	189	20	60	30	96
14	ACDB	152	143	137.5	131.5	125.5	26.5	62.5	53	32
15a	DBCA	196	183	178	174	174	22	62	31	77
17a	CBDA	216	201	192	180	181	35	61.5	60	98
42	CABD	167	157	153	144	142	25	62	32	48
19	DBAC	220	204	198	192	190	30	63.5	43	97
21	DACB	180	170.5	170	166	162	18	60.5	39	00
22	BADC	158	153	148.5	145	142	16	63.5	10	35
46	BCAD	209	199.5	197.5	190	186	23	64.5	44	82
23	CADB	189	184.5	181	172	170	19	63	55	67
24	ADCB	171	160.5	148.5	140	131.5	39.5	63	17	40
Mean values		179.56	168.3	161.3	155.8	151.7	27.80	61.9	34	62.4

Mean weight loss for each month independent of treatment

1st month	11.25 lbs
2nd month	6.98 lbs
3rd month	5.00 lbs
4th month	4.06 lbs

Mean weight loss per month with treatments over 4-month period

Treatment A (placebo)	5.25 lbs
Treatment B (Dextroamphetamine sulphate)	7.09 lbs
Treatment C (thyroid)	0.30 lbs
Treatment D (Dextroamphetamine sulphate + thyroid)	8.05 lbs

TABLE IV

Analysis of randomised block

Source of variation	Sums of squares	Degrees of freedom	Mean squares	Ratio (F)	Probability
Between treatments	161 13	3	53 71	5 4	0 004
Dextroamphetamine sulphate	136 57	1	136 57	12 92	0 0007
Thyroid	24 50	1	24 50	2 31	0 13
Interaction	0 06	1	0 06	0 00	
Between months	687 30	3	229 10	21 67	0 000005
Interaction months × treatments	151 63	9	16 85	1 59	0 13
Between sets of patients*	127 31	5	25 46	2 41	0 14
Residual	792 81	75	10 57		
TOTAL	1920 18	95			

* This source was estimated by dividing the 24 patients into groups of 4, each group forming a Latin square

A χ^2 test was applied to the distribution of the variances for different treatment-month groups of observations and no significant difference from a normal distribution was found

TABLE V

Analysis of 250 other observations

Source of variation	Sums of squares	Degrees of freedom	Mean squares	Ratio (F)	Probability
Between months confounded with treatments	2386 48	10	138 65		
Between treatments	447 30	3	149 10	7 85	0 00007
Interaction	480 41	30	16 01	0 84	
Residual	3925 39	206	19 06		
TOTAL	7239 58	249			

Applying a " t " test to the data for the effects of dextroamphetamine sulphate and thyroid gave a probability of 0 000001 that the effect of dextroamphetamine sulphate was due to chance and of 0 43 that the effect of thyroid was due to chance

(5) The weight lost during the first month was significantly greater than that lost in succeeding months, whichever treatment was used

(6) The rate of weight loss decreased with successive months so that the difference occurring between the second and fourth month was significant, but not between the second and third or the third and fourth This fall in the rate of weight loss with time was neither linear nor logarithmic and did not vary with the treatment used

(7) The mean weight loss per month over the four month period without dextroamphetamine sulphate was 5 77 lbs , dextroamphetamine sulphate increased this weight loss by 2 4 lbs per month, the equivalent of approximately 330 Calories per day excess of energy expenditure over intake
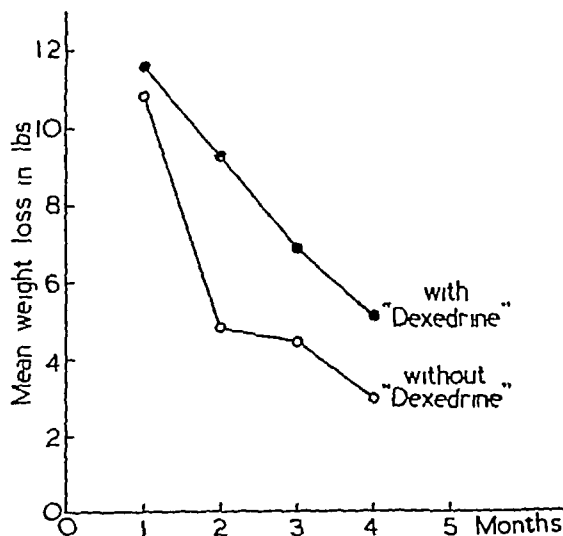
Fig 1 Shows the difference in the weight loss per month when dextroamphetamine sulphate (Dexedrine) is given and also illustrates the change in rate of weight loss with time The dots represent the mean weight loss with treatments B and D, the circles with treatments A and C

Fig 1 illustrates the effect of dextroamphetamine sulphate on the amount of weight lost and the change in the rate of weight loss with time

DISCUSSION

The effect of dextroamphetamine sulphate The experiment was not designed to investigate the mode of action of dextroamphetamine sulphate but some aspects of the results are worth discussing from this point of view

If the main effect of the drug is to suppress appetite it would be expected to increase the weight loss in patients who were otherwise unable to discipline

themselves, without affecting the loss of those with good self-control   The weight losses in the two types of patients would then be more alike than when the drug was not used , in fact the variances of the weight losses with and without the drug were found to be comparable   The lack of a significant difference between these variances *(see* Table IV) might have arisen because of the high level of error and the small number of patients, or because the drug did not act in the expected manner

The experience of the authors is that if the patient succeeds in maintaining a daily deficit of 1,000-1,500 Calories for about two weeks, the appetite decreases considerably and a large proportion of patients who achieve this find the 1,000 Calorie diet adequate to alleviate their hunger   This phenomenon occurs without the use of dextroamphetamine sulphate   After the first month of treatment, therefore, it would be expected that dextroamphetamine sulphate would have less effect upon appetite, yet there is no evidence of such a falling off in the effect of the drug with time

Both these findings suggest that dextroamphetamine sulphate may act in some other manner than by depressing appetite   Most patients did not notice any difference in the effects of the treatments, although a few felt an increased sense of well-being whilst having D and B and some thought that they did not feel as hungry when having these treatments   The opinions of the patients, however, were no reliable guide to the effect of any of the treatments and it seems reasonable to suspect that an important effect of dextroamphetamine sulphate must have been to increase energy expenditure

The change in the rate of weight loss with time   The greater weight loss during the first month was expected in view of the difference in the tissues metabolised   The progressive fall in rate of weight loss over the last three months of the treatment period is unexplained but might be due to any of the following reasons   a progressive increase in Calorie intake , a decrease in surface area and B M R , a decrease in B M R as a compensating mechanism for the Calorie deficit , a decrease in the work done in moving the body as its mass decreases   It is unlikely that there is a steady increase in the Calorie intake in all patients and the fall in rate of weight loss is much greater than the observed change in surface area would produce if the B M R remained normal   Pending further investigation, the phenomenon of decreasing weight loss with time must remain unexplained

SUMMARY

Twenty-four patients were treated for a period of four months with a 1,000 Calorie diet and a placebo, thyroid, or dextroamphetamine sulphate using a randomised block experimental design   Dextroamphetamine sulphate, 5 mg three times a day, was found to have a highly significant action in aiding weight reduction and thyroid B P , 90 mg (grs $\overline{188}$) daily was found to have no significant effect   The weight loss during the first

month of treatment was significantly greater than that in successive months regardless of the treatment used, and the rate of weight loss decreased steadily with time   No definite conclusions were reached as to the mode of action of dextroamphetamine sulphate or the mechanism underlying the progressive fall in the rate of weight loss with time

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THE RELATIONSHIP BETWEEN HORSE DANDRUFF AND HORSE SERUM ANTIGENS IN ASTHMA

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INTRODUCTION

THE study of asthma due to " animal emanations " has made considerable progress since the description of this disorder by Hyde Salter (34) in 1859 In particular, the introduction of skin testing with appropriate extracts based on the original work of Blackley (2) has been invaluable   Asthma due to contact with horse dandruff has been considered specially by many workers ,   the occasional severe shock reactions or deaths following administration of therapeutic antisera to such patients have led to a consideration of these patients' skin sensitivity to horse serum and its constituents (1)   The history of this work has been reviewed elsewhere (24, 4, 36)

Early workers preparing dandruff and other extracts (11, 39, 41) used strong chemical reagents, now known to degrade animal proteins   Grove and Coca (13) introduced simple buffered saline extracting fluids, and claimed to have shown that the allergen in horse dandruff was a protein   Their evidence is equivocal, since digestion with pancreatic ferments apparently in neutral solution at room temperature was employed   As might be expected, even after this treatment of the dandruff extracts used for testing, the skin reactions were positive as illustrated in this account   In general, little is known of the chemical nature of horse dandruff allergens

Animal experimentation has been used in attempts to define the relationship and difference between the antigenicity of horse dandruff extracts and horse serum   In the first successful sensitization experiments (25, 26, 29) anaphylactic reactions in guinea-pigs were obtained with dandruff

extract 3-4 weeks after intraperitoneal injections of this substance   The
reaction was specific, no reactions being obtained to dog and cat dandruff,
nor to horse serum   Later, Forster (9) was able to show cross-reactions
between horse dandruff and horse serum both in guinea-pigs (anaphylactic
reactions) and in rabbits (precipitin tests)   Unfortunately quantitative
techniques were not employed, and the concentrations of the dandruff extracts
used for sensitisation and testing were not measured   Similar criticisms
apply to the studies of Ratner and Gruehl (31) who also elicited anaphylactic
cross-reactions in sensitized animals, and of Tuft (38), who tested sites
passively sensitized with asthmatics' serum in human volunteers

Little fresh direct work on the problems of horse asthma has been
published since 1934   A general statement can therefore be made on the
state of knowledge at this point   Many persons—among them young adults
otherwise in perfect health—are seized with asthmatic attacks when in the
vicinity of horses   These attacks may be so severe as to be disabling for
days at a time   The presence of the living animal is not essential for the
induction of an attack—similar effects may be produced by the clothes of
horse-riders, or even by the small quantities of dust from horse-hair mattresses
or upholstered furniture   Sensitizing antibodies can be demonstrated in the
sera of such persons, especially by injection of small quantities of serum into
normal human skin, thus inducing a local sensitivity   Many tissues are
sensitized in the asthmatic as well as the bronchi—the skin, the conjunctiva,
nasal mucous membrane   Thus, exposure to horse dandruff in the form of
dust may induce conjunctivitis or symptoms of rhinitis   Ingestion of horse
meat may induce urticaria, gastro-intestinal disturbances, or typical asthma
(10)   Similar widespread sensitization may occur in individuals receiving
a blood-transfusion from a horse-sensitive donor

The specificity of the asthmatic's sensitivity is well-marked, so that a
horse-sensitive subject may be able to tolerate all other domestic animals
and even donkeys   On the other hand, a proportion of these patients are
highly sensitive to horse serum, and may also be affected by the dandruff
of other animals—cats, rabbits, etc   Cross-sensitivity to serum has resulted
in deaths and many severe reactions in asthmatics receiving horse serum for
therapeutic purposes, usually with no history of previous serum administra-
tion   These reactions may occur even with purified sera, nominally only
containing globulin, though as will be described later, absolute purity of
such protein fractions is very difficult to attain

The following experiments were intended to elucidate so far as possible
the nature of the substances in horse dandruff and in horse serum causing
reactions in human cases of asthma   Stress has been laid on the quantitative
evaluation of the prick test for human skin testing introduced by Lewis and
Grant (23) and subsequently employed by Hare (14), Harley (17) and others
Antigenicity tests in rabbits were also employed, quantitative precipitin
tests based on the method of Dean and Webb (6) being used   The results

To evaluate the contribution to variance made by sl ʰᶜⁱ
4 concentrations of histamine was pricked into 4 differei   ʼ
tests) on the volar aspect of the forearms of a voluntee
were in imaginary lines on the medial and lateral borders 🌿 ʼ
Each line was divided into 4 sites, the proximal abou
the distal 2″ above the wrist and the others equally ⸲
In this way 16 sites were available for the 16 tests   ⁊
to the 4 histamine concentrations on the basis of a
4 × 4 Latin square (Fisher and Yates (8), Table
16 tests were repeated on the same individual in si
intervals of 48-72 hours to evaluate the contribution
A total of 64 results, i e , 16 with each of the 4 co ⸲
was then available for an⸗   ⸲ ᴐ standard ᵗ
variance was used (e g , Br⸲   ⸲⁊ gave tʼ
III (the full experimental fiᵦ   ⸲   elsewʰ

Analysis ⸲

Concentration of
A = 10 -g /ml
B = 10⁻³g /ml
C = 10 ⁴g /ml
D = 10 ⁵g /ml

Source of variance	sq⸲
Site (rows)	
Days (columns)	6
Histamine concentration (treatments)	191 53
Residual	14 27
Total	217 15

This experiment shows —

(1) A significant source of variance is the site on ⸲

(2) Similarly, there is a significant day to day vⁿ
concentration of histamine

(3) After elimination of these sources of variance, ʰ
determination of wheal size is not greater than √C̄
mm (i e , a coefficient of variation of about 16%)   ⸂
of variation of the 4 concentrations ignoring the site
of variance gave coefficients of variation of 18 3% for
and 23 5% for D   These figures indicate that the cc⁻ᵐᵗᵒⁱ
dependent on concentration, and since the wheal sizes t⸲
over fivefold the sources of variance are probably mainly
technique of measurement   They also show that an aₑ
results if day to day and site to site variations are not taken

discussed below indicate the possibility of preparing therapeutic antisera suitable for administration to asthmatics   Methods of purifying and standardising dandruff protein for skin testing these patients and perhaps for specific treatment are also discussed

EXPERIMENTAL OBSERVATIONS

I   Critical examination of the prick test

The prick test has been widely used and is known to be safe   As the inoculation is very superficial, the test sites do not readily become infected The accuracy of the test has not previously been evaluated quantitatively, nor has the effect of such factors as site-to-site variations in the skin or day-to-day changes in sensitivity been accurately assessed   As considerable reliance was to be placed upon the quantitative results of skin tests with different chemical fractions of horse protein preparations, this precise information was required

Method   A drop of the fluid to be tested is placed upon the skin   A single sharp prick is then made through the drop sufficiently hard to cause momentary pain, but not so deep as to draw blood   After a few seconds, the fluid is dabbed away with cotton-wool, taking care not to contaminate any neighbouring test   For quantitative comparison of the resulting reactions, the wheal and, in suitable subjects, the flare can be measured The standard time for measurement is ten minutes after the prick, as the reaction is then nearly maximal, and yet is still sharply defined   The wheal is traced in outline on to stiff celluloid (*e g*, X-ray film after the removal of emulsion) using Indian ink and a mapping pen   These tracings are enlarged fourfold and the tracing transferred to graph paper using a photographic enlarger   The mean diameter of the wheal may then be calculated accurately after estimating the area of the enlarged tracing by counting squares

Analysis of variance in multiple prick test experiment   Multiple prick tests were carried out using four strengths of histamine as the test substance (It will be seen subsequently that horse protein solutions give similar quantitative relations when prick tested on horse-sensitive subjects' skin) The solutions were made up from histamine acid phosphate to be approximately isotonic to 0 9% saline   The solution was neutralised with caustic soda to pH 7 0 using the glass electrode to determine the amount needed, and then diluted with 0 9% saline to give a 1% (1 100) solution of histamine calculated as free base   From this standard solution, dilutions were made in isotonic neutral buffered saline to contain 1 1,000, 1 10,000 and 1 100,000 parts of histamine   These concentrations afforded a suitable gradation for establishing the dosage-response relationship   Control prick tests through neutral buffered saline gave responses too small for measurement in the subjects studied

Key to Symbols

Concentration of Histamine

A = 10^{-2} g./ml

B = 10^{-3} g./ml

C = 10^{-4} g./ml

D = 10^{-5} g./ml

Lateral   Medial   Medial   Lateral

Fig 1   Allocation of test sites on volar aspect of forearms for analysis of variance in prick tests with histamine

In Fig 2 the mean wheal diameters are plotted against the 4 histamine concentrations, which are shown on a logarithmic scale   The relationship is seen to be approximately linear *   However, the sums of squares shown in Table III for histamine concentrations (3 degrees of freedom) can be broken down to test this relationship with the following result —

TABLE IV

Source of variance	Sums of squares	Degrees of freedom	Mean squares	Significance
Histamine concentrations (treatments)				
Linear	187 01	1	187 01	Very great
Quadratic	3 83	1	3 83	p = 0 001
Cubic	0 09	1	0 09	p > 0 2
	191 53			
Residual (from Table I)	14 27	54	0 264	

It is clear that the relationship over this range shows a significant departure from linearity, as it contains a quadratic component   However, provided that a test solution, $e\,g$, a histamine solution of unknown strength, gives a wheal prick reaction the diameter of which falls within the range tested (1 1-5 7 mm) an approximate value for its concentration can be determined from the graph in Fig 2   The relationship between dose and wheal size has not previously been evaluated for prick testing in man   The approximate relationship in goats was worked out by Darsie *et al* (5) but without adequate statistical analysis

* A similar linear relationship between the diameter of reactions and the logarithm of the concentration of histamine has been found in guinea pigs previously injected with dyes to demonstrate the site of increased permeability (Miles personal communication)   The relationship was also found to hold for certain toxin reactions (28)

To evaluate the contribution to variance made by skin site, each of the 4 concentrations of histamine was pricked into 4 different positions (*i e*, 16 tests) on the volar aspect of the forearms of a volunteer   The sites chosen were in imaginary lines on the medial and lateral borders of the two forearms Each line was divided into 4 sites, the proximal about 2″ below the elbow, the distal 2″ above the wrist and the others equally spaced intermediately In this way 16 sites were available for the 16 tests   The sites were allocated to the 4 histamine concentrations on the basis of a self-conjugate standard 4 × 4 *Latin square* (Fisher and Yates (8), Table XV) as in Fig 1   The 16 tests were repeated on the same individual in similar fashion 4 times at intervals of 48-72 hours to evaluate the contribution of day-to-day variations A total of 64 results, *i e*, 16 with each of the 4 concentrations of histamine, was then available for analysis   The standard technique for analysis of variance was used (*e g*, Brownlee (3)) and gave the results set out in Table III (the full experimental figures are given elsewhere (36))

TABLE III

Analysis of variance in histamine prick tests

Concentration of histamine used	Mean wheal diameter	
A = 10⁻²g /ml	5 71 mm	
B = 10⁻³g /ml	3 82 mm	Mean, all concentrations, = 3 15
C = 10⁻⁴g /ml	2 19 mm	
D = 10⁻⁵g /ml	1 08 mm	

Source of variance	Sums of squares	Degrees of freedom	Mean squares	Significance
Site (rows)	4 94	3	1 65	p = 0 001
Days (columns)	6 41	3	2 14	p < 0 001
Histamine concentration (treatments)	191 53	3	63 84	Very great
Residual	14 27	54	0 264	
Total	217 15	63		

This experiment shows —

(1) A significant source of variance is the site on the forearm where the prick is laid down

(2) Similarly, there is a significant day to day variation in wheal size with a given concentration of histamine

(3) After elimination of these sources of variance, the standard deviation of a single determination of wheal size is not greater than $\sqrt{0\,264}$ mm on an average of 3 15 mm (*i e*, a coefficient of variation of about 16%)   Determination of the coefficients of variation of the 4 concentrations ignoring the site to site and day to day sources of variance gave coefficients of variation of 18 3% for A, 22 3% for B, 22 6% for C, and 23 5% for D   These figures indicate that the coefficient of variation is not greatly dependent on concentration, and since the wheal sizes to which they refer differ by over fivefold the sources of variance are probably mainly biological rather than in the technique of measurement   They also show that an appreciable loss of precision results if day to day and site to site variations are not taken into account

Key to Symbols

Concentration of Histamine

A = 10^{-2} g./ml
B = 10^{-3} g./ml
C = 10^{-4} g./ml
D = 10^{-5} g./ml

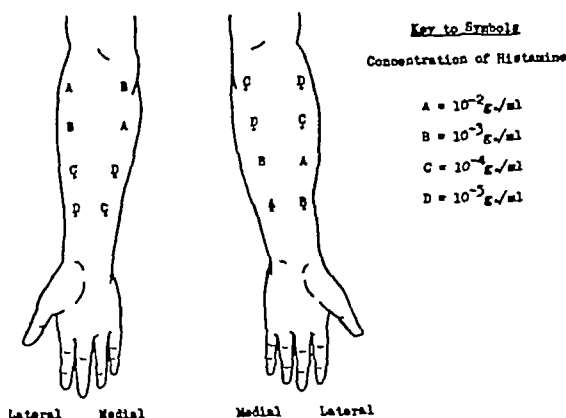Lateral   Medial   Medial   Lateral

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* A similar linear relationship between the diameter of reactions and the logarithm of the concentration of histamine has been found in guinea pigs previously injected with dyes to demonstrate the site of increased permeability (Miles, personal communication)   The relationship was also found to hold for certain toxin reactions (28)

Fig 2   Relationship between wheal diameter and histamine concentration in prick tests   Each point represents the average of 16 readings on the volar aspect of the forearms of a single individual (*see* Table III)

The experiment which has been described did not compare tests with similar concentrations of histamine pricked into symmetrical points on the opposite forearm   A further experiment was therefore carried out to see whether a significant difference existed between such symmetrical sites No significant difference was found   From these two experiments it is concluded that for accurate prick test comparison of two solutions, the tests should be laid down on symmetrical body sites within a short time of one another , the concentrations chosen should be such as to fall within a range over which the relation between dose and wheal size is known (here, wheal sizes of 1-6 mm)   Provided that these precautions are observed, a coefficient of variation of about 16% is to be expected for a single measurement   The order of difference found for tenfold changes in concentration of the test substance was such that these differences could be readily detected with two pairs of observations for each concentration, provided that the precautions considered above were observed

Size of inoculum in prick test   In some of the experiments now to be described it was required to know how large an inoculum was introduced by the prick test   To establish this, symmetrical tests were made comparing the wheal sizes produced by small intradermal injections* (0 001 ml) of very dilute histamine (10^{-5}g /ml) with prick tests using stronger solutions (10^{-3} and 10^{-2}g /ml)   As the intradermal tests gave wheals approximately intermediate in size between these prick tests, it was concluded that the prick test ordinarily introduced 1/300th of the amount injected intradermally, i e , a total of about 3 millionths ml (3×10^{-6}ml)   It is interesting to note

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* *Accurate delivery of 0 001 ml intradermally was made by using a micrometer delivery syringe now available commercially*

that such small quantities can be introduced into the skin with fair precision
The prick test was used throughout the following experiments on human
skin reactions, although in many instances, a more approximate estimate
of wheal size was used—the transverse diameter in two areas at right angles
measured with dividers and scale

*II   Chemical behaviour of horse dandruff fractions, using skin tests to trace
the allergen*

Preparation of dandruff extracts   The crude horse dandruff was obtained
fresh from a serological institute, mixed with hair in the brushings and
combings   The hair was separated by shaking the material with ether, and
pouring the suspension through the holes in a Buchner funnel   The epidermal
scales were then filtered off on filter-paper from the ether (which contained
fat in solution) and rinsed with fresh ether   The powdery grey residue was
dried in an incubator until the smell of ether was no longer detected, and
stored in a vacuum desiccator   To prepare an extract, this dried defatted
dandruff was rubbed up first with toluene and then with normal saline (0 9%
sodium chloride) to give a cream   A little more saline was used to rinse the
material into a flask, and the suspension was left for 3 days at room
temperature (12°-20°C) before filtering   The proportions used in the
extracting mixture were   —dandruff powder 1 g , toluene 1 ml , saline 15 ml
Saline was preferred to Coca's buffered extracting fluid (Coca *et al*, 1931)
as subsequent tests were to include adjustment of acidity

Test subjects for detection of allergens   Two regular subjects were avail-
able for repeated prick testing during the course of the experiments
(Occasionally, the conclusions were confirmed in other patients)   These
subjects were both young men aged between 20 and 30, the one a doctor
and the other a medical student   Both suffered with severe attacks of
asthma as well as with nasal and conjunctival inflammation, in the vicinity
of horses or of materials contaminated with dust derived from horse dandruff,
horse dung, etc   In addition, one subject (J) suffered from hay fever each
summer and gave strong skin reactions when tested with grass pollens ,
the other (T) was sensitive to cats and to certain other animal emanations
Both knew of other sufferers from asthma in his family   The serum from
each individual showed strong sensitising power when inoculated into normal
skin to which it " passively transferred " the appropriate specific sensitivities
These two men were regarded as typical moderately severe cases of asthma,
but were otherwise in normal health   A summary of the case histories of
J and T is given in Appendix I   It seemed preferable to use these two
subjects for repeated testing rather than seek a larger number of patients
with less clearly defined conditions   Any important conclusion was based
on tests in both subjects   It is recognised, however, that the use of two
patients only might in theory lead to generalisations not truly applicable to

all sufferers from horse asthma, and for certain practical applications testing must be carried out on a larger sample

Preliminary examination of chemical properties of horse dandruff extract When prepared in the manner described, the extracts were clear solutions, light brown in colour, tending to froth on shaking   They contained a varying concentration of nitrogen, which was estimated for each extract by the micro-Kjeldhal method (30)   About 2/3rds of this total nitrogen could be precipitated by protein precipitants such as trichloracetic acid (33%) in the proportion of 1 ml to 9 ml of extract   The precipitate and the original extract gave the usual colour reactions of protein solutions (Millon, xanthoproteic, ninhydrin, biuret)   The protein was also completely precipitated by picric acid, tannic acid, or saturated ammonium sulphate   The non-protein nitrogen consisted partly (about 10%) of urea , the remainder was not identified   At least 95% of the non-protein nitrogen was removed after dialysis in cellophane sacs against running water for 3 days—a procedure which also removed much of the brown colouring matter   Dialysed solutions failed to give the qualitative reactions for carbohydrate (Molisch) or for phosphorus

Prick tests in subjects J and T showed that —

   (i) no appreciable loss of activity resulted from prolonged dialysis when allowance was made for volume changes (confirming Coca (4)) ,

   (ii) all activity was removed from solution by saturation~ with ammonium sulphate ,

   (iii) all activity was removed by the other protein precipitants, such as tannic, picric and trichloracetic acids   The reactivity of the protein was not however destroyed by these reagents, as solutions of the precipitates in dilute caustic soda were found to give strong reactions on skin testing ,

   (iv) all active material was thrown out of solution by adding 3 volumes of acetone to 1 volume of extract ,

   (v) the active principle was destroyed by peptic digestion

    Details   Two ml of crude dandruff extract (total nitrogen 92 mg /100 ml) was mixed with 2 ml of a 5% solution of pepsin in saline and 0 14 ml of N hydrochloric acid making the pH 1 5   The mixture was incubated at 40°C for 12 hours in the presence of toluene, along with a control mixture in which saline was substituted for the pepsin solution   After neutralising both solutions, dilutions ranging from 1 5 to 1 5,000 were made from each   The control gave wheals 5 mm in diameter at a dilution of 1 5,000, the digest gave no reaction at 1 5

These results were consistent with the belief that the active substance is a protein   From its solubility in 0 9% saline when preparing extracts and in pure water after prolonged dialysis, this protein is unlikely to be a nucleoprotein or a true globulin—furthermore, insufficient phosphorus or sugar was found for an appreciable content of nucleoprotein to be considered The absence of sugars also showed that mucoprotein was not present in appreciable amounts

Nature of dandruff protein The solubilities of the active dandruff protein in solutions of various salts were consistent with those of an albumin After adjustment of an extract (9 ml) to pH 5 with sodium acetate-acetic acid buffer (1 ml) and adding 2 25 g anhydrous sodium sulphate, the solution was maintained at 40°C for 2 hours A heavy flocculent precipitate formed and was filtered off Analysis showed that about half of the protein remained in solution, and when compared with corresponding dilutions of the original extract no loss of activity was detected The precipitate, after
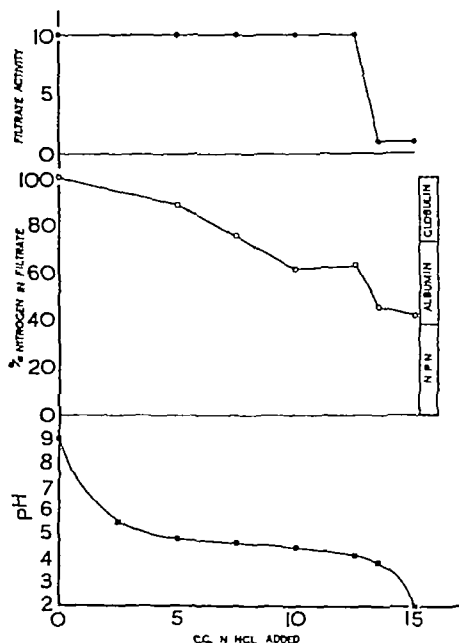
Fig 3 Chart illustrating adsorption of activity and nitrogenous materials from dandruff extract onto benzoic acid crystals at various pH levels

    Method To each of 6 samples of 5 ml. 3N sodium benzoate was added 50 ml dandruff extract N HCl was slowly added —(1) 5 ml (2) 7 5 ml (3) 10 ml (4) 12 5 ml (5) 13 ml and (6) 15 ml Each mixture was well stirred and benzoic acid filtered off The chart shows results obtained with the filtrates

    (a) Activity on skin testing (arbitrary units)

    (b) % of nitrogen remaining in filtrate

    (c) pH

    The block diagram shows for comparison, the fractional nitrogen analysis of the dandruff extract (total nitrogen 31 5 mg /100 ml)

washing with warm saturated sodium sulphate solution adjusted to pH 5, was redissolved and precipitated once more, and washed again On comparing solutions of this precipitate with comparable dilutions of the original extract standardised by nitrogen content, the precipitate was found to be between 5 and 10 times less active This retained activity was considered to be due to adsorption

An attempt was made to concentrate the active fraction still more by adsorption onto a non-specific precipitate (33)   Six mixtures were prepared containing 5 ml 3N sodium benzoate and 50 ml dandruff extract   To each mixture was gradually added, with frequent stirring, normal hydrochloric acid, which produced a finely divided precipitate of benzoic acid crystals   (These crystals form since benzoic acid is much less soluble in water than sodium benzoate)   By adding varying amounts of hydrochloric acid, the adsorption was carried out at various pH values   Skin tests with the supernatant solution from these experiments showed that most of the activity was removed between pH 4 2 and 3 8 (Fig 3)   In a further experiment, therefore, enough hydrochloric acid was added to bring the same dandruff extract-sodium benzoate mixture to pH 4 2   The solution was filtered, the precipitate being discarded, and further hydrochloric acid added to lower the pH to 3 8   On filtration, the crystals were collected and transferred to acetone which dissolved the benzoic acid and freed the adsorbed protein as a precipitate   This protein was collected and made up into aqueous solution   No marked concentration of activity was found to have occurred, and judging from the solubility of this fraction in half-saturated ammonium sulphate, a simple separation of the albumin from the dandruff extract had once more been achieved   This experiment afforded some evidence that the activity of the extracts was not due to some trace substance, but to the albumin fraction itself

An attempt was also made to crystallise the dandruff " albumin " following the usual procedure for preparing serum crystalbumin   An approximate determination of the isoelectric point of the active material was first made by warming 0 10% solutions in M/5 acetate buffer solutions— a flocculent precipitate was obtained, maximal at pH 4   Albumin fractions of extracts were made up in solutions of ammonium sulphate of various strengths and acetic acid cautiously added with frequent stirring until the pH was brought down towards pH 4   No crystalline protein was obtained and it seems probable that some denaturation of the dandruff albumin had occurred, either in the natural drying of the desquamating skin cells or in the original treatment of the dandruff with ether

Effect of heat on dandruff extracts   Autoclaving dandruff extracts at a pressure of 20 lbs /sq inch destroyed about 99% of the activity   Boiling for a few seconds had little apparent effect, but a distinct loss of activity was observed after 30 minutes at 100°C   At pH 5-6, boiling for this period produced a jelly-like coagulum   Adjustment of dandruff extract to between pH 3 and pH 4 produced a flocculent precipitate which increased after boiling the solution for a short period   Activity could be demonstrated in this precipitate by dissolving it in alkali, but the supernatant solution was inactive   It is clear that the active protein can be denatured by such procedures, but the specific groupings of the molecules responsible for its

activity are only gradually destroyed, and so are not of great delicacy
This finding is in accord with the results already described with protein
precipitants

III   The relationship between dandruff and serum proteins found by prick testing

Test solutions and method   The precise relationship between the allergen
in horse dandruff extracts and the constituents of horse serum was next
determined   After having established that each of the test subjects, J and
T , was sensitive to whole horse serum, fractions were prepared as follows —

> Fresh horse plasma was half saturated with ammonium sulphate and after filtering off
> the precipitate (globulins), the albumin was crystallized by cautious addition of acid
> (0 2 N H₂SO₄)   The globulins were redissolved and dialysed for several days against
> running tap water and then against distilled water—a small quantity of thymol was added
> as a preservative   This led to the precipitation of euglobulin, which is insoluble in pure
> water   After filtering off the euglobulin, the filtrate was regarded as pseudoglobulin   To
> purify these three fractions further, the albumin was recrystallised and the globulins were
> separately reprecipitated with half saturated ammonium sulphate three times   Finally,
> each protein was dissolved in 0 9% saline with the addition of ¼% phenol, Seitz filtered,
> and standard strength solutions prepared after micro Kjeldhal estimation of total and
> non protein nitrogen

Prick tests on the skin of each subject were then performed with saline,
histamine solutions, dandruff extract and horse-serum fractions   The results
are set out in Table V

TABLE V

Quantitative prick tests on skin of asthmatic subjects

Test solution	Strength g /100 ml	Subjects			
		J Reaction diameter (mm)		T Reaction diameter (mm)	
		Wheal	Flare	Wheal	Flare
Horse serum albumin	3 5			3	19
	0 5	10	35	2	5
	0 05	7	24	nil	nil
	0 005	5	19	nil	nil
Horse serum euglobulin	0 5	4	15	nil	nil
	0 05	3	9	nil	nil
	0 005	nil	nil	nil	nil
Horse serum pseudoglobulin	3 5	6	26	nil	nil
	0 5	5	20	nil	nil
	0 05	5	12	nil	nil
	0 005	3	9	nil	nil
Horse dandruff protein (unfractionated)	0 001	6	26	12	35
	0 0001	3	5	5	27
	0 00001	nil	nil	3	20
Histamine phosphate	(expressed as base)				
	1 0	6	25	8	40
	0 1	4	10	6	30

Each result represents the arithmetical average of four measurements made on paired
tests   Control tests of all solutions in skin of normal subjects gave wheals less than 2 mm
and no flares   Similar reactions in above tests are recorded as "nil"   All the above
measurements were made 15 minutes after prick.

From Table V a number of results and conclusions have been drawn

(1) Readily detectable reactions were seen in each subject to high dilutions of dandruff protein (0 0001g /100ml in J , 0 00001g /100ml in T) Since the prick test only introduced about 3×10^{-6} ml , the total amount of dandruff protein required to elicit a detectable wheal in T was of the order of 10^{-13}g (Assuming a molecular weight for the protein of about 100,000 this quantity is equivalent to 10^{-18} gram molecules, $i e$, only about 10^5 molecules)

(2) In each subject, a comparison of the reaction elicited by histamine with those by dandruff protein showed that similar sized results were only obtained with histamine solutions about 1,000 times stronger Since the molecular weight of the dandruff protein is probably about 1,000 times greater than that of histamine (100,000 as compared with 100), if the dandruff reactions are mediated by histamine, then each dandruff molecule must be supposed to liberate about 1,000,000 histamine molecules This liberation must also be supposed to occur very rapidly—say within one half minute This consideration might suggest that the H-substance of Lewis is not histamine itself, but is some pharmacologically more active substance in promoting wheal formation

(3) Neither test subject showed as great a sensitivity to any horse serum fraction as to unfractionated horse dandruff protein

(4) Of the three horse serum fractions which were tested, albumin gave the largest response in each subject In the strengths of 0 5g /100ml , for example, subject T only responded to serum albumin In this and lower strengths, subject J responded most strongly to serum albumin , the reaction to euglobulin and to pseudoglobulin being explainable if these solutions were contaminated with albumin to the extent of about 0 1%, and 1% respectively Evidence will be presented later to show that this order of contamination was in fact present

(5) The results set out in Table V also showed that the active wheal-promoting material in dandruff extract and serum albumin could not be a single substance common to each For whereas subject T reacted more strongly than subject J to a given strength of dandruff, he reacted less strongly than J to a given strength of serum albumin The results can most simply be regarded as examples of cross-reactions to serum albumin, which is, on this hypothesis, antigenically related to but not identical with dandruff albumin The cross-reaction hypothesis accords also with the fact that the cross-reactions to serum albumin were weaker than to the allergen in dandruff extracts

Tests with special horse serum fractions Horse serum albumin has been separated by Hewitt (18, 19, 20, 21, 22) into carbohydrate-free crystalline albumin, named " crystalbumin," and into carbohydrate-rich fractions known as " seroglycoid " and " globoglycoid " with higher molecular

weights It seemed desirable to determine whether the most highly purified crystalbumin available would produce reactions when pricked into the asthmatics' skin Dr Hewitt kindly supplied three horse serum albumin fractions —

1 Crystalbumin which had been recrystallized twelve times

2 A solution rich in seroglycoid

3 A solution rich in globoglycoid

The seroglycoid and globoglycoid could not be regarded as free from crystalbumin The three fractions were each diluted so as to contain 0 5% protein and tested in subject J

TABLE VI

Test solution		Strength g /100 ml	Subject J	
			Reaction diameter (mm)	
			Wheal	Flare
1	Crystalbumin	0 05	5	20
2	Seroglycoid	0 05	4	18
3	Globoglycoid	0 05	4	10
			Each result repre sents the average of four measure ments	

The largest response was given by the highly purified crystalbumin The reactions given by globoglycoid- and seroglycoid-rich solutions were *consistent with albumin contents of between* 10% *and* 20% *concentration which according to Dr Hewitt might well have been present* Although the exact nature of globoglycoid and seroglycoid is unknown, and the difference between globoglycoid and crystalbumin even disputed (Rimington and Van den Ende (32)), these results show that *crystalbumin itself gives a cross-reaction in the horse asthmatic skin test* Comparison of Tables V and VI show a somewhat weaker reaction with the highly purified albumin, but as the tests were not performed in parallel, this should not be taken as definite evidence of a loss of potency in the purified product In other experiments with fresh horse serum and similar material stored in sealed ampoules with cresol for prolonged periods, loss of activity could be shown in the stored material by skin testing

Cross-reaction between dandruff protein and serum albumin in passive transfer experiment Six sites on the skin of a normal volunteer were inoculated intradermally with serum (1/20 ml /site) from subject J After

24 hours, two sites were pricked with horse dandruff extract (0 45%), and two with horse serum albumin (2%)   In each case a typical wheal (7-8 mm in diameter) and flare (25-35 mm in diameter) appeared   After a further lapse of 24 hours, the remaining two sites were prick tested, one with horse dandruff extract, the other with horse serum albumin , wheals and flares were obtained as on the previous day   But the sites already tested reacted neither to serum albumin nor to dandruff extract   The experiment was repeated on another normal volunteer with similar results   It was concluded that passively sensitised sites could be made to produce a full reaction and desensitised either by dandruff extract or by serum albumin   This conclusion provides further evidence for the view that the asthmatic's sensitivity is to a single dandruff protein of albumin type, which is antigenically related to serum albumin   (This experiment does not confirm the findings of Tuft (38), who considered that horse dandruff and horse serum showed specific desensitising effects   In Tuft's experiments, greater sensitising doses of serum from patients were used , this leads to a larger area of sensitised skin so that desensitisation is a less precise procedure)

IV   Antigenic relationship between dandruff and serum proteins in the rabbit

The advantages of the quantitative precipitin reaction, using the method of optimal proportions (Dean and Webb (6)) have already been mentioned, and this method was used throughout in the following experiments   Rabbits were injected with horse dandruff extracts or with horse serum and its fractions, and precipitating antisera produced   The specificities of these antisera were tested toward the dandruff and serum proteins   In some experiments, the specificity was more exactly defined by adsorption of some of the precipitin components

Methods   Four Angora rabbits, each weighing about 6½ lbs , were given intensive courses of injections as shown in Table VII   In each case, the first two injections were intraperitoneal, while subsequently the intramuscular route was used   Periodically, rest intervals were allowed in which no injections were given   Blood was withdrawn towards the end of these periods to assess the progress of precipitin production   Final bleedings were made two weeks after completing the injection courses   The rabbit injected with dandruff extract received a smaller dosage (reckoned as total protein) than the other animals, since the extracts contained a lower concentration of protein than the serum preparations   No rabbit was given the serum pseudoglobulin as this preparation was judged to contain too great an admixture of the other proteins to produce an even relatively specific antiserum

For the titration of an antiserum, 0 5 ml amounts of graded antigen dilutions were placed in suitable dilution in small test tubes (⅜″ in diameter) and 0 5 ml quantities of antiserum were added rapidly to each   The mixtures were placed in a water-bath which was thermostatically maintained

TABLE VII

Time and dosage used for immunisation of rabbits

Animal	Material injected	Total duration of immunisation	Total dosage of protein
1	Horse serum	17 weeks	1 9 g
2	Serum albumin	11 weeks	1 1 g
3	Serum euglobulin	11 weeks	0 44 g
4	Dandruff extract	8 weeks	0 14 g

at 40 °C and fitted with a glass face   The tubes were illuminated from above and observed continuously so that early signs of precipitate formation could be readily seen as an opalescence   The optimum mixture of antigen and antiserum was determined from the tube in which a granular precipitate first appeared   If, in another experiment, the antiserum was further diluted, then the optimum concentration of antigen was correspondingly reduced , the optimal proportion remained the same though the precipitate took longer to form and was diminished in amount   It was found convenient to use antiserum dilutions such that a granular precipitate was formed in about 30 minutes, and in this way, mixtures containing the optimal proportion could readily be distinguished from mixtures differing twofold in antigen concentration

Antigen and antiserum dilutions were always allowed to warm in the bath before mixtures were made   Accurate and rapid delivery of antigen was made from a 2-way tap connecting a reservoir with a syringe clamped so as to fill to the 0 5 ml mark , on turning the tap this amount could be expelled into the antigen tube   In the bath, the level of the mixture in the tubes was maintained a little above the surface of the water so that convection currents kept up a gentle circulation in each tube   In preliminary titrations of each antiserum, a series of antigen dilutions differing 10-fold in strength were used   The final results were obtained using suitable 2-fold dilutions of antigen mixed with a dilution of antiserum chosen so as to give precipitates in about 30 minutes   For adsorption tests, suitable dilutions were rapidly mixed, maintained at 40 °C for 2 hours and then allowed to stand 12 hours at 5 °C   before centrifugal separation of the flocculent precipitate

Results and conclusions   The results obtained are set out in Table VIII, in which each experiment is numbered for convenience in reference

Experiments 1-4 showed that the antiserum produced by injecting unfractionated horse serum gave precipitates not only with serum fractions but also with dandruff

TABLE VIII

Quantitative precipitation reactions of rabbit antisera

Experiment No	Antiserum from rabbit injected with	Antigen	Dilution of antiserum	Precipitate + or 0	Optimal antigen concentration g /100 ml
1	Horse serum	Serum albumin	1 50	+++	0 001
2	,, ,, ,,	Serum euglobulin	1 10	+++	0 01
3	,, ,, ,,	Serum pseudoglobulin	1 50	+++	0 25
4	,, ,, ,,	Dandruff extract	1 50	+	0 2
5	Serum albumin	Serum albumin	1 50	+	0 002
6	,, ,,	Serum euglobulin	1 50	0	—
7	,, ,,	Serum pseudoglobulin	1 50	++	0 5
8	,, ,,	Dandruff extract	1 50	++	0 2
9	Serum euglobulin	Serum albumin	1 10	+++	0 02
10	,, ,,	Serum euglobulin	1 10	+++	0 05
11	,, ,,	Serum pseudoglobulin	1 10	++	0 01
12	,, ,,	Dandruff extract	1 10	++	0 2
13	Dandruff extract	Serum albumin	1 2	+	0 02
14	,, ,,	Serum euglobulin	1 2	0	—
15	,, ,,	Serum pseudoglobulin	1 2	0	—
16	,, ,,	Dandruff extract	1 2	+	0 2
17	,, ,,	Serum albumin	1 10	0	—
18	,, ,,	Dandruff extract	1 10	+	0 04
19	Antiserum to horse serum 1 5 adsorbed with equal amount serum albumin 0 013%	Serum albumin	1 10	0	—
20		Serum euglobulin	1 10	++	0 025
21		Serum pseudoglobulin	1 10	0	0 1
22		Dandruff extract	1 10	0	—

Note —Range of antigen dilutions tested —

	Protein concentration
Serum albumin	0 2–0 00002 g /100 ml
Serum euglobulin	0 5–0 0005 g /100 ml
Serum pseudoglobulin	0 5–0 0005 g /100 ml
Dandruff extract	0 5–0 00025 g /100 ml

Experiments 5-8 show further that the antiserum to horse serum albumin also precipitated both serum albumin and dandruff protein   This antiserum failed to precipitate serum euglobulin in the range of concentrations tested (shown at foot of Table VIII) but produced a precipitate when mixed with the preparation of pseudoglobulin   (Reasons will be given below for assuming that this effect was due to albumin remaining in the pseudoglobulin solution)

Experiments 9-12 indicate that the euglobulin solution also contained some albumin, for the antiserum to euglobulin gave precipitates both with albumin and with pseudoglobulin   The amount of a precipitin produced is known to bear no simple direct relationship to the amount of antigen injected, so that no further conclusion can be drawn from this antiserum

Experiments 13-18 show that the antiserum produced by injecting dandruff extract gave precipitates both with dandruff and with serum albumin, but not with the serum globulins in the concentrations tested Again, then, the cross relationship between dandruff and serum albumin is confirmed   Some individual specificity for the antigen injected dandruff extract is evident in *Experiments* 17 *and* 18 which show that on further dilution, the antiserum failed to precipitate serum albumin while still being active against dandruff extract

For *Experiments* 19-22, the antiserum was the same as that used in *Experiments* 1-4   In this series, a preliminary adsorption had been carried out by admixture of serum albumin   As a result, the antiserum though still capable of precipitating either of the globulins, gave no precipitate either with serum albumin or with dandruff extract   This effect is the converse of the observations made in *Experiments* 5-8 *and* 13-16

To understand the precipitate formed in *Experiment* 7, when pseudo-globulin was mixed with "antialbumin," the quantitative results shown in the last column must be considered   Since the antiserum (at a dilution of 1 50) gave an optimal precipitate with 0 002% albumin (*Experiment* 5) and with 0 5% pseudoglobulin (*Experiment* 7) the observations are consistent with a proportion of albumin of $\frac{0\ 002}{0\ 5} \times 100 = 0\ 4\%$ of the total protein present in the pseudoglobulin preparation   A similar proportion (1%) was postulated on p 138 when discussing the results of skin testing the human subjects   Evidence that this explanation is correct is afforded by further experiments —

(1) The same sample of pseudoglobulin purified to a smaller degree by two, instead of four, precipitations with ammonium sulphate reacted optimally with the "antialbumin" at a concentration of 0 05%   This is consistent with 4% of the less highly purified pseudoglobulin preparation being albumin

(2) After addition of excess albumin to the " antialbumin," a further precipitate could no longer be obtained on admixture with pseudoglobulin

(3) After adsorption of the " antialbumin " with 0 5% pseudoglobulin, no further precipitate could be obtained on admixture with albumin

The specimen of euglobulin, though containing traces of the other serum proteins as shown by *Experiments* 9-12, could not have contained much albumin   Comparing *Experiment* 5 with *Experiment* 6 (in which 0 5% euglobulin was the highest concentration tested) less than $\frac{0\ 002}{0\ 5} = 0\ 4\%$ of the protein was made up of albumin   Again referring to p 16, skin testing suggested the presence of about 0 1% of albumin in the euglobulin preparation used   Clearly, the complete removal of traces of a soluble protein like albumin from the precipitates used to prepare the globulins is a matter of some difficulty

The precipitin reactions which have been described prove an antigenic relationship between horse serum albumin and horse dandruff protein, and fall into line with the chemical results and the skin test findings given in previous sections   *Experiments* 17 *and* 18 demonstrate the individual specificity of " antidandruff " serum   Analogous specificity was found for the " antialbumin "   For whereas adsorption of " antialbumin " with serum albumin removed all precipitins both for albumin and for dandruff extract, adsorption of " antialbumin " with dandruff extract failed to remove all the antibodies which could precipitate albumin in spite of complete removal of its activity against dandruff   Quantitative measurements in this experiment showed —

(1) Admixture of dandruff extract removed about 75% of the " antialbumin " as judged by the fall in optimal concentration of serum albumin for precipitation with this adsorbed serum

(2) Using two samples of 5 ml " antialbumin " serum, *A* and *B*, *A* mixed with albumin, *B* with dandruff extract, each in optimal proportions, more protein was precipitated with the direct reaction with *A*, than with the cross-reaction with *B*   The amounts obtained from micro-Kjeldhal analysis of the precipitates were —*A* (albumin-antialbumin mixture)—1 4 mg protein , *B* (dandruff-antialbumin mixture)—0 9 mg protein

These experiments strongly support the hypothesis that the antigenically active protein in the dandruff extracts is an albumin, related to but not identical with serum albumin   The alternative hypothesis that dandruff extract contains a mixture of antigenically active, unrelated proteins with some admixture of serum albumin was considered and rejected since many of the results obtained would be difficult to interpret on this basis   Nor has any evidence in favour of this more complex possibility been discovered (as, for instance, a double optimum in the precipitin reactions between dandruff extract and the antidandruff serum)

DISCUSSION

Dandruff and serum albumin significance of relationship   The existence
of two proteins closely related in chemical and serological properties has been
discovered in the dandruff albumin and serum albumin of the horse, and
has not, so far as is known, been previously described   Such relationships
are recognised among proteins of different species (*e g*, hæmoglobins), but
when proteins from different sources in a single species have been studied in
a sufficiently pure state, they have always been found to be distinct (*e g*,
hæmoglobin, serum albumin, serum globulins)   The distinction between
serum albumin and the globulins has once more been confirmed in this work
but the well-known clinical relationship in asthmatic patients between
dandruff and serum sensitivity has been found to be due to an antigenic
relationship between dandruff and serum albumin   Nor does the relationship
rest upon some peculiarity of the human allergic state, since analogous
cross-reactions were found in the sera of rabbits immunised with serum
albumin and with dandruff extract

Such a finding deserves further study by accurate chemical definition
of the dandruff protein in terms of its amino-acid composition and physical
characteristics (as defined, for example, by electrophoresis and ultracentrifuge
experiments)   Most of the work which has been described here was carried
out in isolation during 1942-3, and could not then be extended in such
directions   Further studies are now planned, but some speculation as to
the possible biological significance of the findings already available seems
justified

The presence of an albumin in the desquamating skin cells of the horse
might be due to local synthesis by the skin of a protein similar to that turned
out by the liver* into the circulation as serum albumin   Alternatively,
serum albumin itself might escape from the circulation and be taken up by
the skin cells to be used as a cellular component after suitable modification
The latter hypothesis seems the simpler, and is in accord with recent work
(40, 7) showing that serum albumin freely leaves (and indeed re-enters) the
circulation   From isotope studies (35), tissue constituents can no longer
be regarded as isolated from the general bodily economy, receiving simple
nutriments and returning even simpler waste products—even complex
materials like protein molecules are in a constant state of exchange flux
From its small size, albumin is the most likely plasma protein constituent
to enter into this cellular exchange   The precise contribution which might
be made by such a protein within the cell can at present only be a matter of
surmise, but there is some evidence to show that protein synthesis is generally
accompanied by linked protein breakdown   Thus, it is possible that the
production of specialised proteins (*e g*, keratin) depends upon the energy
liberated from the breakdown of albumin   Again, albumin has certain

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* The liver is regarded as the most likely source of serum albumin although this hypothesis
still awaits unequivocal proof

special properties which enable it to form lipoid and carbohydrate complexes which may be of importance within the cell as well as in the circulating plasma   Such suggestions as these are to be regarded as a basis for further experiment rather than as anything approaching firmly proven fact

It is important also to avoid reading more into the experimental findings than is justifiable   The absence from dandruff of antigenic globulin (as shown by the rabbit experiments) may indicate the absence of this type of protein in the skin cells of the horse   But equally, the drying undergone by the desquamating epithelium and the use of ether in defatting the scales before making extracts may have rendered such material insoluble and prevented its appearance in the extracts   The antigenic difference of dandruff albumin from serum albumin may similarly have been an exhibition of slight denaturation rather than some specific functional alteration of this protein in the skin cells of the horse

The ability of albumin to withstand drying and other treatment without becoming insoluble or much degraded is no doubt related to its role as a sensitiser in asthma   It seems probable that the allergens of grass pollen (15) and of ragweed pollen (37), for instance, are albumins also, so that the finding for horse dandruff is not unique   Whether the tendency for asthmatics to become sensitive to various species of animal should be regarded as an example of cross-reactions to partially denatured albumins (in which species specificity is known to be somewhat reduced) can only be decided after large-scale quantitative skin testing with a wide variety of allergens

Such general considerations and the unanswered questions which they evoke must not be allowed to distract attention from immediate practical problems—the diagnosis, severity grading, possible treatment of the asthmatic patient and provision of sera suitable for administration to asthmatics   Some observations arise from this work under each of these headings

Specific diagnosis and severity grading of asthma   Until *in vitro* serological methods are devised, specific diagnosis in asthma must rely upon careful skin testing   For this method to be efficient, allergen preparations of known potency must be available   Simple nitrogen determinations of dandruff extracts are not adequate as a basis for standardisation since a variable quantity of non-protein nitrogen is present in the preparations   Protein nitrogen would be a better basis, and better still, would be a purified albumin fraction prepared from the filtrate after saturation of the extract with sodium sulphate   Although such purified extracts, Seitz-filtered to obtain sterility, and stored in sealed ampoules are probably fairly stable, the experience with serum albumin (p 139) suggests that the only ultimate standard of potency must be a quantitative human biological assay, comparing extracts for issue with a freeze-dried standard   The quantitative

skin testing methods which have been described in this report provide a basis for such work, and indicate the number of tests which would be required for any given level of reliability   Such standardised preparations could be used for grading asthmatics in severity, for following the development of sensitivity (a process about which little is known) and for studying the disappearance (or masking) of sensitivity achieved by treatment

Specific treatment of asthma   Considerable success has followed the administration by injection of graded doses of pollen extract to hay-fever patients, though the mechanism underlying the results is still disputed (27, 16)   Similar results are possible with horse dandruff extracts (*see*, for instance, early results obtained with horse serum by Goodale, 12), though it is likely that up to the present many physicians have been deterred from attempting such treatment by the lack of suitable clean, standardised allergen preparations   These can now be prepared and standardised in the manner suggested   Administration of dandruff protein will have to be made with great care, as shown by the minute dosage producing marked skin reactions   Preliminary protection of the patient with full dosage of the new " antihistamine " drugs would minimize reactions to the allergen (Harley (17))   and it is possible that such a method would greatly accelerate specific treatment

Antisera suitable for administration to asthmatics   On occasion, the sufferer with horse asthma needs serum treatment for unrelated conditions   For example, a severely contaminated wound may make the administration of anti-tetanic serum desirable   If skin tests carried out on a large series of horse-sensitive asthmatic patients confirms the finding that they are not affected by the globulin fractions of horse serum, methods of producing albumin-free antisera should be adopted   It has been seen that successive precipitation with ammonium sulphate can reduce the albumin content of globulin fractions to less than 0 5% of the total protein   Antisera which would be safe to inject into severe horse-sensitive asthmatics would need to contain very much less than this amount   But serum albumin and serum globulin differ sufficiently in chemical nature for more efficient methods of separation to be devised, and for a safe antiserum to be attainable   If possible, such preparations should then be used universally, since severe reactions still occur in patients who are unaware of their horse sensitivity, or who fail to disclose it   These antisera could not be guaranteed not to produce reactions in patients previously injected with horse globulins, but it is likely that accidents originating in this way are relatively rare, and seldom as severe as the reactions seen in asthmatic patients

SUMMARY

1   Sensitivity to horse emanations is frequently accompanied by sensitivity to horse serum, so that severe reactions may follow administration of antisera prepared from horses   This paper describes experiments made to define the relationship between horse dandruff and horse serum antigens

2   Experimental observations were made on two horse-sensitive asthmatics using the prick method of skin testing   The reliability of this method was established by repeated testing with histamine and subjecting the results to analysis of variance   The prick test was found to introduce about 3×10^{-6} ml into the skin

3   Quantitative prick tests were used as a basis for studying the chemical nature of the antigen in horse dandruff extracts, which was found to have the properties of an albumin

4   Further tests showed that the asthmatic subjects were also sensitive to horse serum albumin, including highly purified preparations of cryst-albumin   Other horse serum proteins appeared to give no effects except those due to contamination with serum albumin   Quantitative considerations showed that the reaction to horse serum albumin was best regarded as an immunological " cross-reaction " due to the relationship between serum- and dandruff-albumin rather than to the presence of a common substance in serum and dandruff

5   Precipitating antisera were obtained in rabbits by injection of horse dandruff and of horse serum proteins   Again antigenic relationships were found to exist between horse dandruff protein and horse serum albumin   Adsorbed sera and quantitative estimations of precipitate formation with mixtures of antigens and antisera in optimal proportions gave further support to the " cross-reaction " concept

6   The implications of these findings have been discussed   The presence of an albumin in the skin cells of the horse probably derived from serum albumin suggests that cell proteins may not be entirely built up in each tissue from amino-acid synthesis   Practical implications for diagnosis, severity grading and treatment of asthmatic patients are discussed   Anti-sera such as antitetanic serum could probably be made safe for administration to horse-sensitive asthmatic patients if they were completely freed from horse serum albumin

APPENDIX I

Notes on history of subjects used in skin tests

Subject T   (*Medical practitioner*)
Age when tested—29 (*now* 35)

History

Suffered from broncho pneumonia at age of 5   When 6 years old, asthma attacks were noted after contact with horses and cats   Cat scratches induced whealing and intense itching Horse dander also produced rhinitis and conjunctivitis (horse reactions thought to be more rapid at onset and more severe than those due to cats)   Skin testing at age of 6 confirmed sensitivity to horse and cat extracts   Sensitivity to dogs also noticed after age of 23 after receiving " desensitising " injections containing mixed animal hair and feathers

Has also suffered from hay fever (vasomotor rhinitis) every summer from May to September for many years, and gives positive skin reactions to grass pollen extracts   Mild eczema, mainly on soles of feet, between ages of 5 and 7

Asthmatic attacks have been recurrent since childhood, with no tendency to become milder When living in the country, patient takes aspirin nightly to avoid being awakened by bronchospasm   In town, this may not be required, but after contact with animals, ephedrine or adrenaline is required to control attack

Severe attacks of asthma have followed contact with sister's riding clothes Following
intradermal tests on one occasion local and generalised reaction followed including asthma,
rhinitis, conjunctivitis and œdema of arm and face, and needing adrenaline administration
Subject was unable to complete " house appointments " in hospital owing to cat asthma, and
chose career as pathologist to avoid living in hospital or visiting patients' homes

Family history

Mother—hay fever (died with disseminated sclerosis)

Father—eczema late in life (died pulmonary phthisis)

Paternal uncles—? asthma (detailed history not available)

Sister—alive and well—no allergic symptoms

Treatment tried has included " desensitising " injections and, recently, " antihistamine " drugs
without success aspirin has been found useful to control mild attacks, ephedrine or adrenaline
for severe attacks

Subject I   (Medical student—now practitioner)
Age when tested—22 (now 29)

History

In infancy (' age 2-4) subject suffered with tonsillitis and adenoids Tonsillectomy was
performed was followed by broncho pneumonia and then by whooping cough   " Bronchitic "
attacks persisted and were only gradually recognised as bronchial asthma

Definite sensitivity to horses was present at age 4, *e.g* , after riding, wheezing forced subject
to return home Severe urticaria once noticed at a " meet "

Sensitivity to dogs was suspected at age 8 9 following close contact, but has never been
as marked as horse asthma

Hay fever and summer asthma (June September) started at age of 7 9 No evidence of
sensitivity to other farm animals (*e g* , bullocks and sheep)

Since childhood, has suffered with recurrent mild attacks of asthma nearly every night
unless ephedrine is taken Often worse June September especially if subject is in the country
Free from attacks for a whole month once when at sea Bad attacks also follow upper respiratory
tract infection two or three times each winter

Family history

Father died cardiac asthma (no allergic history)

Paternal grandmother—asthma from age of 1 till death at 65

Mother—alive and well—no allergic history

Treatment

Ephedrine for control of attacks , apparent benefit from desensitisation injection courses
with pollens , breathing exercises

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THE ACTION OF NORADRENALINE IN MAN AND ITS RELATION TO PHÆOCHROMOCYTOMA AND HYPERTENSION

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THE chemical structure and pharmacological activity of several sympathomimetic amines were investigated by Barger and Dale (7) who pointed out that the actions of adrenaline and the effect of sympathetic nerve stimulation were not always similar and that the latter often resembled more closely the action of amino ethanol catechol (noradrenaline) They also demonstrated that the latter substance usually had a distinctly greater pressor activity Interest in the subject of sympathetic mediators was revived by the demonstration by Euler (17) of a substance isolated from sympathetic nerves with actions closely resembling those of noradrenaline More recently Gaddum and Goodwin (21) found that the substance released on stimulation of the hepatic sympathetic nerves (liver sympathin) had similar properties to those of noradrenaline, and Peart (34) has reached similar conclusions for splenic sympathin Bulbring and Burn (12) have shown that noradrenaline is released from the normal adrenal gland

The widespread existence of noradrenaline in the body and the demonstration of its presence in a phæochromocytoma removed from a patient with hypertension who came under our observation have led us to examine the circulatory actions of noradrenaline in human subjects and to consider how far they resemble the changes seen in hypertension Since this work was begun, investigations on noradrenaline in man have been reported by Goldenberg and others (22), Barcroft and Konzett (6), and Swan (43)

PART I   THE ACTION OF NORADRENALINE ON NORMAL MAN

Methods

Synthetic lævo-noradrenaline, provided through the kindness of Dr Tainter of the Winthrop Chemical Co , was used in most experiments The

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racemic mixture (d-1) was also used and had about half the activity of the pure lævo form   The pure substance was prepared in 1 1,000 solution according to the B P formula for adrenaline injection,* and sterilized by autoclaving   Assays by Mr J J Brown on the cat showed that the solution kept in the refrigerator for three weeks, the longest period before use, showed no loss in activity

Throughout each experiment saline was infused through a needle inserted into a vein of the arm or leg   The noradrenaline solution was injected through a side arm of the system by a mechanically driven syringe delivering 1 ml per minute   In each observation the subject received saline, noradrenaline and saline in successive periods   The general appearance of the subject, the pulse rate and the blood pressure were noted and in some cases electrocardiograms were taken

In some experiments the arterial pressure was measured directly from the femoral artery by a capacitance manometer   An 18 gauge Record needle was inserted into the arterial lumen under local anæsthesia and was connected by a three way tap to the manometer incorporated in the hydraulic system without intervening tubing   Photographic records were obtained from a cathode ray oscillograph connected to the electrical system of the manometer   The natural frequency of the system including the needle was about 100 cycles per second   The instrument was calibrated against a mercury manometer at the beginning and end of each experiment   This type of instrument was chosen in preference to a membrane manometer of the Hamilton type because it can be incorporated directly in the needle system   The wide rigid lead piping which should be used if a membrane manometer is to retain its more desirable physical properties can thus be discarded   Furthermore, the capacitance manometer is readily assembled for use and there is no complex system of mirrors and lenses to manipulate

Heat elimination from the hand of the warm subject was measured in a Stewart calorimeter modified by Greenfield and Scarborough (28)   The forearm blood flow was estimated by the venous occlusion plethysmograph with the surrounding water bath maintained at 34°C   The plethysmograph was fitted close to the elbow to include as much muscle as possible and the lower forearm and hand flow was excluded by a cuff

Inulin and diodone clearances were determined by the procedure and methods of analysis described by Sanderson (40) with the exception that inulin was estimated by a diphenylamine method without preliminary yeast treatment   The procedure was as follows   2 5 ml. of plasma filtrate or diluted urine were heated with 6 ml of diphenylamine reagent in a boiling water bath for 12 minutes, and cooled in running tap water for 4 minutes   The resulting coloured solutions

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* Formula

Noradrenaline	0 1 g
Tartaric acid	0 08 g
Sodium metabisulphite	0 1 g
Sodium chloride	0 8 g
Water for injection to 100 ml	

were compared with standards in a photoelectric absorptiometer, using Ilford 604 filters (max transmission 520 mμ) Proteins were removed from plasma by the cadmium hydroxide method (20) using 1 in 10 dilution The diphenylamine reagent was prepared by dissolving 2 7 gm diphenylamine in 90 ml glacial acetic acid, and when solution was complete adding 60 ml concentrated hydrochloric acid This solution was prepared freshly on each occasion. Standards were made from a stock inulin solution on each occasion the standards for plasma were prepared by taking 1 ml lots of plasma drawn before the inulin infusion began adding known amounts of inulin and then precipitating the proteins with cadmium hydroxide By heating for only 12 minutes the plasma blank colour is greatly reduced much more so than the colour due to inulin and in our experiments the plasma blank colour was generally equivalent to about 4 mg inulin/100 ml The plasma inulin levels employed ranged from 28 to 40 mg /100 ml It was thought that the infusion of noradrenaline might vitiate the inulin figures through a rise in the blood sugar and consequent rise in the plasma blank colour However analyses of a series of blood samples drawn before, during and after noradrenaline infusion in the absence of inulin, indicated that variations in the plasma blank colour due to this cause were insignificant In all experiments except No 33 the noradrenaline infusion was begun immediately after the end of the second clearance period but the third period was not begun until the infusion had been running for ten minutes the urine collected during these ten minutes was discarded The noradrenaline infusion was stopped at the end of the third period and was followed by another ten minute discard period In experiment No 33 no discard periods were interposed and the second period coincided exactly with the noradrenaline infusion

Most of the subjects were healthy male students, but a few infusions were also given to some older patients free from circulatory disease   For comparison 9 infusions of commercial l-adrenaline were given   This preparation was not assayed for noradrenaline, but it may have contained a variable amount of noradrenaline (4)   The adrenaline used for the intra-arterial records was synthetically prepared

The effects of noradrenaline infusions

Subjective sensations   With the smaller doses of l-noradrenaline (1-15 μg per min) the majority of subjects had no abnormal sensations   A few noted a sense of constriction behind the sternum associated with an increased awareness of respiration, but this passed off in most subjects within a few minutes   Some complained of mild palpitations, but this was uncommon With larger doses up to 30 μg per minute all these symptoms were slightly more frequent   Only one subject complained of headache , his blood pressure at the time was 190/120   Others with a similar degree of hypertension were unaffected   It should be emphasised that all the above sensations were mild and in no way interfered with the performance of the experiments

General appearances   Within about 1½ minutes of beginning the administration of noradrenaline a definite blanching of the face, lips and mucous membranes of the mouth and a less obvious pallor of the limbs developed and persisted throughout the infusion   On stopping the infusion there was a feeling of warmth and a pronounced flushing of the face appeared and persisted for a few minutes

The mucous membrane of the ileum seen in an ileostomy paled during an infusion of 25 μg per minute   The rectal mucosa seen through a procto-scope also paled slightly during an infusion of 25 μg per minute   Intradermal injection of 0 1 ml of 1 1,000 to 1,000,000 dilution of l-noradrenaline produced local intense pallor and gooseskin   Injection into the mucous membrane of

the ileum also produced local pallor   The veins near the infusion needle constricted with noradrenaline, at times so intensely as to stop the infusion

In two subjects receiving 30 μg l-noradrenaline per minute for 16 and 40 minutes respectively, a soft swelling in the region of the thyroid gland developed   In the subject receiving the shorter infusion the swelling subsided within an hour   In the other, at the end of the infusion, the neck measured 44 cm in circumference and after six hours resumed its normal measurement of 39 cm   In some subjects a similar, but much less conspicuous swelling was observed   In none of the subjects receiving noradrenaline alone was any venous congestion noted in the neck
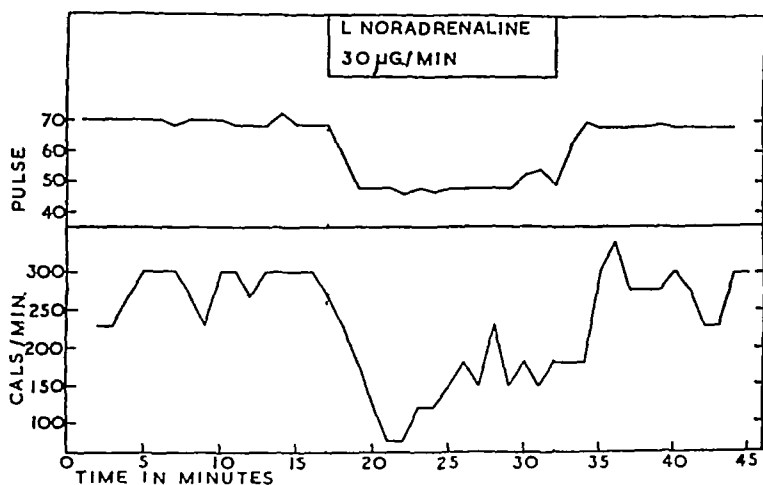
Fig 1   The effect of noradrenaline on the heat elimination from the hand

Pupil   No changes in the size of the pupil were seen during infusions at a rate of up to 30 μg per minute or from dropping of l-noradrenaline in 1 1,000 solution into the conjunctival sac

Pulse rate and blood pressure   Our results confirm those of all other workers in showing that infusions of noradrenaline in the doses used invariably produced a rise in systolic and diastolic blood pressure, and a slowing of the pulse (Table I-III)   The results obtained with the capacitance manometer will be described later

Heat elimination from the hand   Table I summarises the observations In eleven the heat elimination fell during noradrenaline to rise again after its termination   A representative result is shown in Fig 1   In three there was a progressive rise or fall during the observations   The constrictor effect of noradrenaline was, as anticipated, most easily demonstrated when the hand vessels were dilated,

TABLE I

Blood pressure, pulse rate and heat elimination from the hand before during and after noradrenaline infusions in normal subjects

Expt No	Subject	Age	Weight kg	Dose µg/min	First control period			Noradrenaline infusion			Second control period		
					B P	Pulse	H.E cal/min	B P	Pulse	H E cal/min	B P	Pulse	H.E cal/min
1	O B	52	58	10 dl	—	70	95	—	64	87	—	72	114
2	G K	42	70	15 dl	114/77	75	217	134/85	65	152	119/70	78	224
3	T O C	43	71	15 dl	112/62	60	380	112/68	61	280	110/66	70	294
4	A S	44	66	20 dl	—	85	360	—	66	203	—	83	202
5	A S	39	66	10 l	110/78	70	312	130/100	57	242	113/76	78	330
6	O B	52	58	15 l	145/85	75	107	205/115	58	114	147/80	75	213
7	A H	30	70	15 l	104/70	60	290	130/100	46	152	110/68	60	171
8	J T	22	60	20 l	137/70	67	330	156/107	82	200	130/80	92	374
9	A B	31	76	30 l	131/93	74	107	143/105	62	175	120/91	74	210
10	L F	38	63	30 l	108/68	92	270	147/84	53	233	111/71	84	312
11	O S	27	67	30 l	129/90	75	151	157/112	53	232	130/65	74	445
12	G M S	22	61	30 l	127/86	77	407	151/106	62	210	128/70	73	351
13	H P	22	69	30 l	110/54	69	285	155/97	40	148	120/54	68	278
14	W D	21	80	30 l	132/80	60	410	201/112	42	152	135/80	73	260

Forearm blood flow   Table II summarises the results in 10 observations
In all the subjects the blood flow was reduced   In two cases the alteration
was slight   When a marked depression of the blood flow developed there
was a transient hyperæmia on stopping the noradrenaline (Fig 2)

Renal clearances   The results are summarised in Table III, and the
changes in one experiment are seen in Fig 3   It will be seen that the usual
changes in blood pressure and pulse rate occurred in each experiment   The
inulin clearance showed little change except in experiment 31, in which it
fell by 18% from the values without noradrenaline (average of periods 1, 2
and 4)   In the remaining subjects it either fell very slightly or showed no
significant change   The average change induced by noradrenaline in all
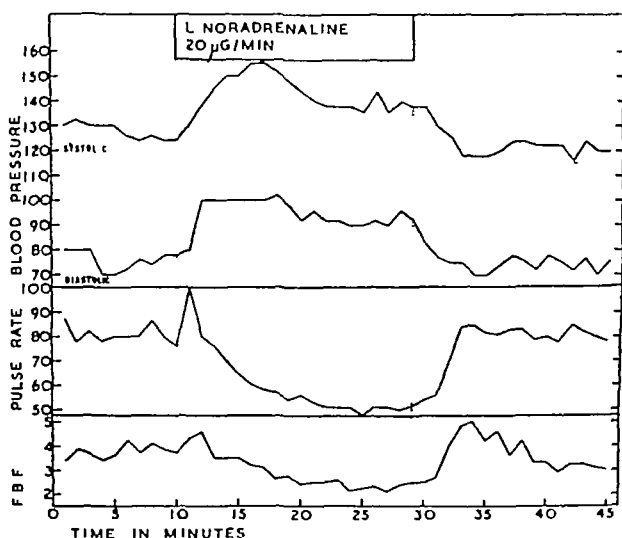
Fig 2   The effect of noradrenaline on the forearm blood flow (F B F)

experiments was —5%   On the other hand, the diodone clearance was
considerably depressed in all six experiments   The largest deviation from
the control value was —31% and the smallest —12%, the average deviation
being —26%   It follows from this that the filtration fraction (CIN/CD)
showed a consistent elevation with noradrenaline   The largest increment
was 8 0, the smallest 2 9 , the average in the 6 experiments was 5 4

The urine flow was recorded in experiments 32 and 33 , there was a
suggestion of a fall during the noradrenaline infusions but the changes were
slight and probably not significant   In experiment 35 where no catheter
was used, a water diuresis was induced to aid voluntary emptying of the
bladder   The fall in urine flow in period 3 is more likely to have been
part of the normal subsidence of the diuresis than an effect of noradrenaline
since the effect is still more marked in the two control periods which followed

The considerable inhibition of diuresis seen in period 4 was probably of psychic origin as there was an extraneous disturbance at the time   In the subsequent period the diuresis made a considerable recovery towards its former level
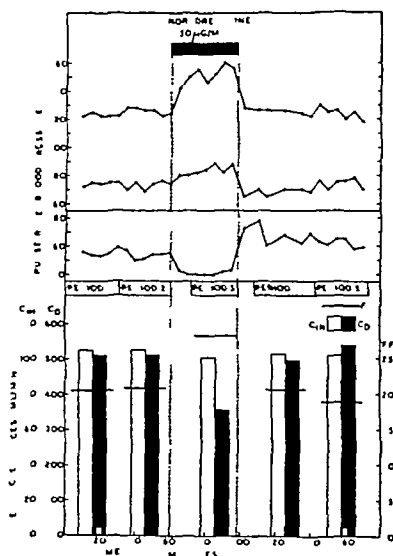
Fig 3   The effect of noradrenaline on renal clearances

Intra-arterial pulse records and a comparison of the actions of adrenaline and noradrenaline

The results obtained by measuring the arterial pressure with the sphygmomanometer by the auscultatory method suggest that the actions of adrenaline and noradrenaline on the circulation differ in a material way   Thus it seems that noradrenaline raises the diastolic pressure while adrenaline often leaves this value unchanged or reduced   But it is sometimes found in experiments with adrenaline, as in patients with aortic regurgitation, that there is no abrupt fading of the sounds as the pressure in the cuff is gradually lowered and the observer is naturally led to inquire how valid are the indirect readings of arterial pressure under these grossly abnormal states of the circulation

Considerations such as these led us to obtain readings of systolic and diastolic pressure directly with a capacitance manometer   Three subjects were given 30 µg l-noradrenaline per minute and the changes in blood pressure and heart rate recorded continuously   The experiment was then repeated giving 30 µg l-adrenaline per minute   The results are summarised in Table IV and the changes observed in experiment 38 are illustrated in

TABLE II

Blood pressure, pulse rate and forearm blood flow before, during and after noradrenaline infusions in normal subjects

Expt No	Subject	Age	Weight kg	Dose µg/min	First control period			Noradrenaline infusion			Second control period		
					B P	Pulse	Blood flow ml/100 ml/min	B P	Pulse	Blood flow ml/100 ml/min	B.P	Pulse	Blood flow ml/100 ml/min
15	G P	45	78	25 dl	108/68	65	3 4	123/81	65	3 0	116/78	69	3 3
16	B B	22	73	30 dl	120/53	85	4 5	135/84	61	3 0	125/73	82	4 0
17	O.B	52	58	151	152/92	70	4 1	200/115	55	3 2	145/90	68	4 6
18	J B	27	63	201	148/90	81	3 6	172/103	69	3 4	148/90	84	4 4
19	A.D	28	59	201	118/79	70	1 6	138/96	50	1 5	118/83	70	1 6
20	A P	31	57	201	128/76	81	3 7	138/94	52	2 4	122/76	82	3 2
21	H P	22	59	201	128/70	62	3 6	150/100	52	3 5	130/78	66	3 6
22	L H	21	70	301	120/78	80	3 5	144/108	54	3 1	120/70	80	4 0
23	J G	22	69	301	124/83	72	3 4	188/117	52	3 4	132/92	68	4 0
24	D H	21	67	301	115/65	69	3 2	180/110	30	2 0	120/65	70	3 1

TABLE III

Blood pressure, pulse rate and renal clearances before, during and after noradrenaline infusions in healthy males

Expt No	Subject	Age	Weight kg	Period	1 noradrenaline µg/mn	Duration minutes	B P	Pulse	Urine flow ml/mn	Inulin clearance ml/mn	Diodone clearance ml/mn	Filtration fraction %
30	H L	32	70	1	0	18	112/77	00		100	570	18 3
				2	0	15	108/75	07		88	402	18 5
				3	20	14 5	124/87	54		80	304	22 5
				4	0	14 5	110/77	04		90 5	505	16 1
31	P	22	72	1	0	21	132/00	07		101	704	20 2
				2	0	18	130/90	04		150	826	10 2
				3	25	15	100/101	40		120	562	22 4
				4	0	17	138/00	08		142	745	10 1
32	R	22	86	1	0	10 5	130/05	74	1 9	102	745	21 8
				2	0	10	130/80	73	2 0	155	790	10 7
				3	30	14 5	101/108	47	1 0	145	545	20 6
				4	0	10	138/03	72	1 0	140	710	10 7
33	R B	20	73	1	0	23	120/83	73	4 5	110	704	15 2
				2	30	25	168/104	51	3 7	111	474	23 1
				3	0	22	140/83	76	5 7	95	523	18 2
34	P S	32	77	1	0	18	135/77	78		133	407	28 5
				2	0	17	130/73	78		148	522	28 4
				3	20	19	144/85	58		144	450	31 4
				4	0	16	130/71	73		145	572	25 3
35*	P S	32	73	1	0	31	123/74	54	10 0	105	511	20 0
				2	0	20 5	125/74	52	15 5	107	512	20 0
				3	30	26	155/83	41	11 5	101	357	28 3
				4	0	28 5	125/08	70	3 5	103	497	20 7
				5	0	30 5	124/74	62	0 0	102	540	18 0

* Done without using an indwelling catheter To induce diuresis 1000 ml water were drunk during the 90 minutes preceding the first period A total of 1200 ml were drunk during the five periods

Fig 4   In all cases the response was similai   Infusion of noradrenaline led to a progressive and steady rise in systolic and diastolic pressure and a coincident slowing of the heart   The pulse pressure was increased (Fig 5) The changes with adrenaline were more complex   Approximately three quarters of a minute after beginning the adrenaline infusion there was a conspicuous, but fleeting, fall in the systolic and diastolic pressure and a considerable cardiac acceleration (Fig 6)   Thereafter, the systolic pressure rose above, and the diastolic to about the same level as before the infusion was begun   In two experiments (36 and 38) the pulse returned to the previous rate but in experiment 37 there was considerable slowing throughout
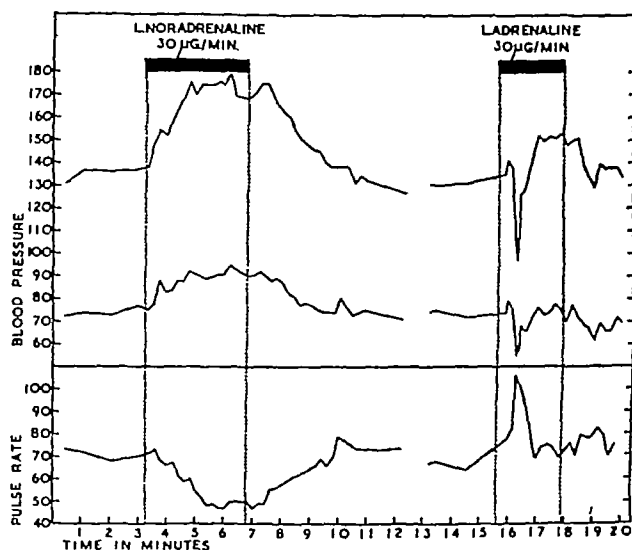
Fig 4   The effect of noradrenaline and adrenaline on pulse rate, and blood pressure   Calculated at 15 sec intervals from the continuous intra-arterial tracings in experiment 38

the remainder of the infusion   The resting pulse rate in this subject was, however, unusually rapid   This type of response with adrenaline has been reported by previous workers (25, 36)

Other differences between the two substances were also observed Infusions of adrenaline were accompanied by readily noticeable symptoms such as conspicuous trembling and palpitations even when the dosage was low   Noradrenaline in comparable amounts produced little in the way of symptoms   We have also confirmed the observations of Allen and others (3) on the effect of intravenous infusion of adrenaline on forearm blood flow At the onset of the infusion at a period corresponding in time with the great fall in blood pressure there was a transitory enormous increase in blood flow which, however, subsequently settled down at about twice the previous resting level   The action of noradrenaline and adrenaline on the heat

TABLE IV

Blood pressure and pulse rate determined by capacitance manometer before, during and after noradrenaline and adrenaline infusions in normal subjects

Expt No	Subject	Age	Weight kg	Drug and dose per minute	Control period		Infusion of drug				Control period	
							After 45 sec		Rest of infusion			
					B P	Pulse	B P	Pulse	B P	Pulse	B P	Pulse
36	A P	31	60	1 noradrenaline 30 µg	135/82	74	155/92	95	195/110	91	140/78	70
				1 adrenaline 30 µg	137/83	80	120/80	105	150/82	70	120/72	80
37	H P	25	70	1 noradrenaline 30 µg	134/82	92	150/92	80	100/120	56	135/84	94
				1 adrenaline 30 µg	132/82	98	103/62	120	175/92	75	134/78	98
38	A S	30	75	1 noradrenaline 30 µg	135/74	70	155/85	66	173/91	48	130/74	75
				1 adrenaline 30 µg	130/78	65	105/00	100	153/80	73	130/70	75

elimination of the hand was approximately similar and intradermal injection of the two drugs in increasing dilutions produced blanching and goose-skin in about equal degree

The nature of the bradycardia

In the majority of the subjects receiving the smaller doses of noradrenaline the rhythm during the infusions was slow, but regular   The
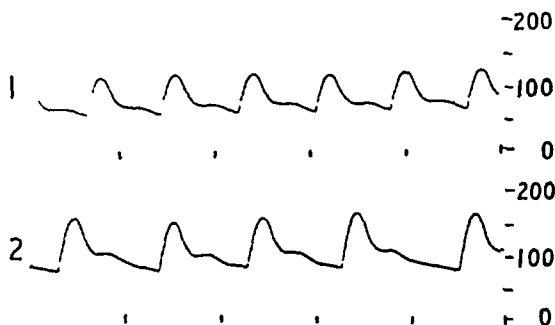
Fig 5   Arterial pulse tracings from experiment 36   1   Before   2   Three minutes after beginning 1 noradrenaline infusion 30 µg /minute   Time interval one second , pressure in millimetres of mercury
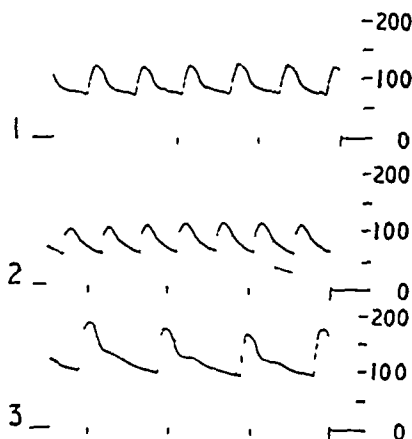
Fig 6   Arterial pulse tracings from experiment 37   1   Before   2   45 seconds after beginning an infusion of 1 adrenaline 30 µg /minute   3   Two minutes later   Time interval one second, pressure in millimetres of mercury

intra-arterial records showed that the hypertension and bradycardia developed together and there was no evidence that the fall in pulse rate ever preceded the rise in pressure   Electrocardiographs showed a sinus bradycardia with or without lengthening of the PR interval   With larger doses, occasionally an irregular rhythm was observed and in two subjects this disturbance was conspicuous   In one the electrocardiogram showed the development of complete AV dissociation, but the records taken immediately before an

infusion of 30 μg per minute of noradrenaline showed bundle branch block
presumably of congenital origin, though the heart was clinically normal
In the second subject l-noradrenaline at the rate of 15 μg per minute led to
sinus bradycardia (Fig 7)   At a rate of 30 μg per minute there was a com-
plete suppression of normal sinus activity and the development of ventricular
complexes of nodal origin   Coupling of the beats also occurred and in this
subject at a later date these changes were reproduced by digital compression
of the carotid sinuses for 10 seconds   These findings suggested that the
bradycardia was a vagal reflex from the carotid sinus and aortic arch   To
investigate this idea further the response of five subjects to noradrenaline
was studied both before and after the intravenous administration of atropine
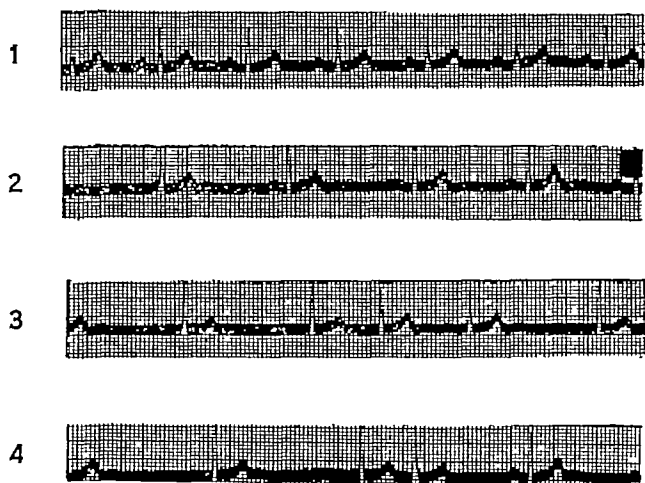
Fig 7   Electrocardiograms—all lead 2   1   Before   2   During l noradrenaline 15 μg /minute
showing sinus bradycardia   3   During l noradrenaline 30 μg /minute showing bradycardia,
irregular nodal beats and suppression of P waves   4   During bilateral carotid sinus
pressure

in doses of from 1 2 to 2 1 mg (Table V)   The pulse rate after atropine varied
in different subjects from 84 to 108   In all a slight transient reduction in
heart rate was noted at the onset of the noradrenaline infusion, but this was
quickly followed by an increase in heart rate either up to or above the
previous level, and at the same time there was a great rise in arterial pressure
The dosage of l-noradrenaline was restricted to 10 μg per minute, but even
this amount, which only produced a slight rise before the administration of
atropine, led to extremely high pressures in some atropinised subjects   On
stopping the noradrenaline, the blood pressure fell rapidly, but the heart
rate declined more slowly   In one subject noradrenaline after atropine
produced a rise in venous pressure, seen in the neck veins , noradrenaline
alone produced no recognisable rise   The forearm blood flow was measured

in three of these experiments   In one the results were inconclusive owing
to a steady decrease in the values obtained without noradrenaline   In the
other two noradrenaline after atropine led to an increase in the blood flow
(Fig 8)

The bradycardia was considered to be vagal in origin consequent on the
rise in intravascular pressure, for the following reasons   The continuous
intra-arterial records showed that the slowing developed coincidentally with
the rise in pressure and that in no case did the bradycardia precede the
hypertension   The electrocardiographic changes were similar to those
described by Lewis (32) following excessive vagal activity, and their
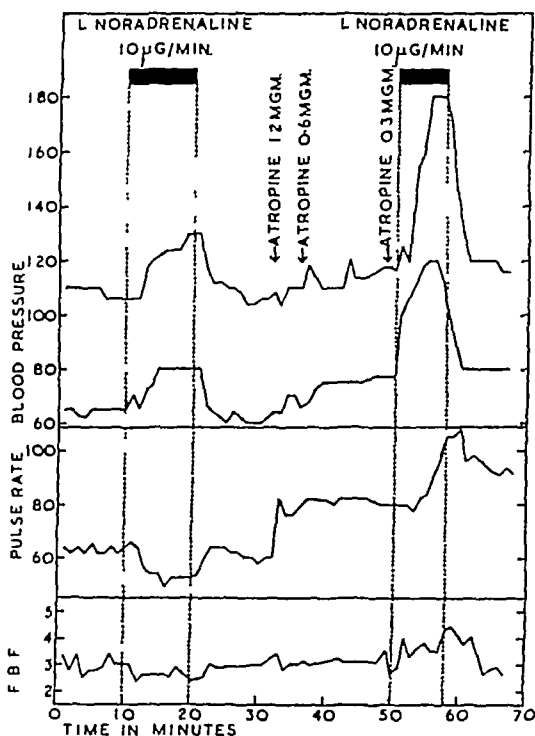
Fig 8   The effect of noradrenaline and intravenous atropine sulphate on blood pressure, pulse
rate and forearm blood flow

reproduction in one subject by carotid sinus pressure supported this view
The evidence with atropine was in conformity, for the bradycardia was very
largely eliminated   It is true that in each experiment the onset of the
effects of noradrenaline was associated with a transient and slight slowing
of the heart , but the heart rate following the injection of atropine was
such as to suggest that the vagus was not completely paralysed   After the
initial bradycardia noradrenaline quickened the heart of the atropinised
subject, an effect which may be explained in one of two ways   First,

TABLE V

Blood pressure, pulse rate, and forearm blood flow during noradrenaline infusions before and after the administration of atropine in normal subjects

Expt No	Subject	Age	Weight kg	l noradrenaline dose μg per min	Atropine dose	First control period			Noradrenaline infusion			Second control period		
						B P	Pulse	Blood flow ml/100 ml/min	B P	Pulse	Blood flow ml/100 ml/min	B P	Pulse	Blood flow ml/100 ml/min
25	D S	27	73	10	1 2 mg	115/70 148/100	48 84		120/90 184/136	40 98		118/76 138/06	47 80	
26	T H	27	83	10	1 2 mg	110/76 118/80	60 90		124/90 200/150	46 108		114/78 112/80	64 96	
27	W P	42	67	10	2 1 mg	108/65 114/70	64 82	3 0 3 1	125/80 180/114	52 96	2 0' 3 0	100/62 110/80	62 88	2 0 2 0
29	D B	22	75	10	1 2 mg	130/08 142/88	62 108	8 2 8 7	154/80 176/108	57 110	5 7 7 3	142/08 130/80	60 114	8 0 6 4
20	G P	45	78	10	1 5 mg	118/73 134/04	62 08	2 1 3 2	132/02 180/110	56 08	2 3 4 1	128/80 118/82	60 96	2 7 2 9

noradrenaline is known to accelerate the isolated mammalian heart (2) In normal human subjects acceleration is not seen, presumably on account of the reflex vagal inhibition   With partial paralysis of the vagus by atropine the direct action of noradrenaline on the heart may be more evident Secondly, one of our subjects given atropine and noradrenaline showed a rise in venous pressure and although we made no direct measurements, it is quite possible that an increase in right auricular pressure may occur and lead to cardiac acceleration   It is probable that the combination of the two drugs leads to a considerable increase in cardiac output accounting for the greater forearm blood flow and the enormous rise in blood pressure

Discussion

The findings reported above are in general in agreement with the results obtained by other workers   Goldenberg and others (22) and Barcroft and Konzett (6) have both reported the rise in systolic and diastolic arterial pressure and the bradycardia   The latter workers have also demonstrated decreases in calf blood flow on infusions of noradrenaline into the femoral artery   The conspicuous blanching from intravenous as well as intracutaneous injection shows that noradrenaline has a powerful constrictor action on the minute vessels of the skin and mucous membrane of the mouth and to a lesser extent on that of the ileum and rectum   The decrease in hand blood flow suggests but does not prove, that the arterioles may also be affected   The decrease in forearm flow despite the rise in arterial pressure suggests that the drug constricts muscle vessels, its action in this respect being in striking contrast to adrenaline   The renal circulatory changes induced by noradrenaline are similar to those caused by adrenaline and other pressor agents, such as ephedrine, paredrinol and angiotonin (5, 11, 38, 41)

The swelling of the neck, thought to be due to enlargement of the thyroid gland, has not been previously reported during experimental noradrenaline infusions   A similar swelling has, however, been noted during paroxysms of hypertension due to a phæochromocytoma (42) though we did not observe it in any of our cases

PART II   PHÆOCHROMOCYTOMA AND THE ROLE OF ADRENALINE
AND NORADRENALINE

During recent years increasing attention has been paid to the diagnosis and treatment of tumours of the adrenal medulla, and many reviews of the condition have appeared (10, 15, 16, 33)   Few investigations, however, have been made regarding the circulatory disturbances associated with the condition and the mechanism of the development of the paroxysmal and persistent forms of hypertension   The following three cases were studied chiefly from these points of view

Case 1   W H , a male of 38 years was in good health until May, 1946, when he gradually developed increasing anorexia, lassitude and a feeling of

faintness   The blood pressure at that time was 180/110   Subsequently he noticed that he was sweating a great deal and had occasional attacks of dizziness and headaches   In January, 1948, he began to lose weight and to develop attacks of epigastric discomfort, followed by extreme pallor, vomiting, shivering, shortness of breath, profuse sweating and the development of " goose-flesh " all over his arms   The pallor was particularly noticeable and was observed as a warning signal of the approach of an attack   From the onset of these attacks he noticed that he was unable to appreciate temperature differences with his right hand, and he occasionally burnt his right fingers with his cigarettes without noticing the heat   He developed a particularly severe attack on the 20th July, 1948   That morning he went to work feeling rather dull and a bit hazy in his eyes and could not see well   He began to feel weak, nervous and irritable   He was extremely pale   He went home to bed and there he had severe continuous pain in the epigastrium and in the loins

He was admitted on the same day to Chase Farm Hospital under Dr T Simpson and on examination there his hands and face were cold and clammy, the fingers cyanosed and the face very pale   There was profuse sweating over the whole of the body and his pyjamas were changed six times during the night   He was very anxious, there was gooseskin flesh over the extremities and his hair was standing on end   He was restless and shivering, his teeth were chattering and he thought he was going to die   The blood pressure was 200/140, and there was obvious papillœdema in both eyes with hæmorrhages and exudates   His symptoms gradually subsided and subsequently, after a mass had been felt below the right costal margin, a diagnosis of phæochromocytoma was made and he was transferred to St Mary's Hospital on 28th August, 1948, under the care of Prof G W Pickering

Except for rheumatic fever when 10 years old, the patient had previously been healthy   His father died aged 45 years after an operation for appendicitis   His mother died aged 60 years from some cause unknown to the patient   One sister died of high blood pressure in childbirth aged 20 years   One brother, aged 42 years, has a peptic ulcer   His blood pressure was 120/80 in November, 1948

The patient was a thin, nervous individual with a moist skin and a course tremor of the hands   Venous congestion of 2 cm above the sternal angle was present in the neck   The pulse was regular, usually about 100 The blood pressure was 170/115 , pulsus alternans was observed in the range of 170 to 160 mm   The cardiac apex beat was visible over a large area, moving the ribs and palpable 12 cms from mid-line in the 5th space   Gallop rhythm was present but no murmurs were heard   In the abdomen a mass with a firm rounded lower edge was felt deeply below the right costal margin moving with respiration   In the right hand he was unable to discriminate

hot and cold up to the level of the wrist joint   The nervous system was otherwise normal, but the optic fundi showed bilateral papilloedema, small hæmorrhages and soft white exudates   There was a distinct right macular fan of silvery exudate and a slight left fan

Urine —albumen nil, sugar nil, deposit nil   Urea concentration test — maximum 3 6 gm /100 ml   Blood urea 34 mg /100 ml   Hæmoglobin 15 6 gm /100 ml   W B C   14,000   per   cmm   Polymorphs 62% Eosinophils   1%   Lymphocytes   36%   Monocytes   1%   Serum potassium 19 2 mg /100 ml   Serum cholesterol 210 mg %   Normal glucose tolerance curve   B M R + 20%   E C G —Left axis deviation with slight ST depression and inversion of T in all limb leads   Intravenous benzodioxane test as described by Goldenberg and others (22) produced a rise in blood pressure from 185/125 to 225/130   Perirenal insufflation of air (by Mr Goligher) and radiological examination (by Dr E Rohan Williams) showed clearly a right suprarenal tumour

At operation, on 23 9 48, Mr A Dickson Wright removed a tumour of the right suprarenal gland measuring 5 cm in diameter and weighing 45 gms   The opposite suprarenal gland was normal and no tumours were found elsewhere   Pathological examination (Dr R C B Pugh) showed the tumour to be a phæochromocytoma occupying the medulla of the gland with thinned cortex stretched over it   The tumour was soft, lobulated, pinkish grey in colour with areas of old and recent hæmorrhage   Microscopically, the tumour was composed of irregular polygonal cells of varying size and shape, with frequent mitotic figures   The nuclei were vesicular and the cytoplasm contained granules staining brown with chromic salts   The tumour was encapsuled   A renal biopsy was examined microscopically in serial sections   Many glomerular afferent arterioles showed irregular and scattered areas of hyaline change in their walls, but no fibrinoid degeneration was seen   Ischæmic changes as evidenced by thickening of Bowman's capsule or hyalinisation of the tuft were present in a few glomeruli   The majority of the glomeruli were of normal appearance

After the operation he had no further attacks   The papilloedema rapidly subsided and the other abnormalities in the fundus gradually disappeared   Normal sensation returned to the right hand after four weeks In the electrocardiogram the left axis deviation persisted but T1 and T2 became upright and the ST depression disappeared   When last seen in December, 1949, he felt entirely fit and had been regularly at work   He still complained of slightly defective vision in the right eye especially noticeable when reading   The fundi appeared entirely normal

The circulatory changes before and after operation are shown in Fig 9 The blood pressure remained persistently elevated after operation and in December, 1949, it was 170/122   The forearm blood flow varied but the changes were not correlated with the blood pressure   The changes in the

ma\imal heat elimination of the hand (35) were most striking, the noteworthy feature being failure of the vessels to dilate while the tumour was present The heat elimination remained at an extremely low level, although every effort was made to produce full reflex vasodilatation by placing the opposite forearm and hand in stirred water at 45° centigrade   The patient sweated profusely during these investigations   After excision of the tumour the ma\imal heat elimination rose to a level found in normal subjects by Pickering (35)

A histamine test in December, 1949, carried out as described by Roth and Kvale (39) produced no paroxysm of hypertension
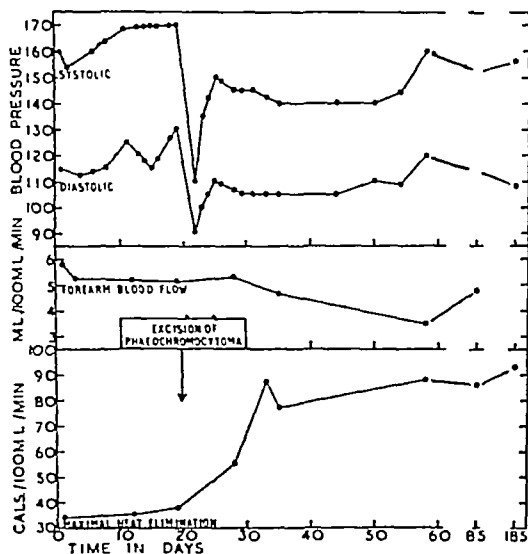
Fig 9   The effect of excision of the phæochromocytoma in Case 1

An infusion of l-adrenaline at the rate of 20 μg per minute before operation produced trembling, pallor and tachycardia   The patient stated that the sensations during this infusion were similar to those experienced in an attack

Case 2   Mrs R B , aged 49 years, first complained of early morning headache in 1944 which lasted from a few hours to the whole day and was sometimes accompanied by nausea, vomiting and profuse sweating   These symptoms became worse   Since 1946 breathlessness on hurrying and swelling of the ankles in the evening were also noted   She had no nocturnal dyspnœa but sometimes woke at night with attacks of palpitation, thumping at the back of the head and profuse sweating   Her appetite had always been good, but she lost 28 lbs in weight in the last 2 years   She had occasional epigastric

pain one hour after food, relieved by alkali or vomiting, but was little troub by this   Her bowels were regular and she had no urinary symptoms was becoming increasingly nervous

In November, 1945, she attended the Royal Bath Hospital because headache, shortness of breath and swelling of the ankles   At that time ι blood pressure was 220/130, the heart was stated to be moderately enlarg the fundi were normal, the urine showed no albumin and the blood urea ʌ 30 mgm %

In November, 1947, she again visited the Royal Bath Hospital blood pressure was 220/98 and two smooth swellings found under the coʌ margins were thought to be the liver and spleen   A barium meal shcw no intrinsic lesion of the stomach, which appeared to be displaced forwʌ and to the right by a grossly enlarged spleen   The urine contained a trʌ of albumin and a few epithelial cells   A blood count was normal

Her mother died at 71 years from cerebral thrombosis   Her father alive at 74 years and is stated to have high blood pressure which does nʌ trouble him   There are three brothers, two sisters and one son all ah and well

She was admitted to St Mary's Hospital under the care of Dr W D Brooks on 20th September, 1948   She was a ruddy faced, middle agʌ woman of average build   The pulse was 120 and regular   The bloc pressure was 200/130   There was a dorsal scoliosis, concave to the Lʌ The apex beat was in the fifth left intercostal space, 4″ from the mid lin and the sounds were normal   In the fundi were a few retinal hæmorrhagʌ some soft exudates and early arteriovenous nipping   There was ι papillœdema   The liver was enlarged two fingers' breadth below the r g costal margin and its edge was firm   There was a smooth tumour moʌ ι with respiration below the left costal margin   The nervous system wʌ normal

Radiological examination (Dr E Rohan Williams)   the chest showeι no pulmonary lesion   The heart appeared slightly enlarged to the left, ʈ the transverse diameter was within normal limits   Excretion urograɪ ʲ ʎ showed normal kidneys   A large mass was present lying in front of ʌ ι left kidney, but not of true renal origin

Blood urea was 40 mgm per 100 ml   Urea concentration test ʌ ʌ ιι ι 2 3 gms urea per 100 ml urine   Blood Wassermann and Kahn reactioʌ negative   Blood hæmoglobin 12 gms per 100 ml   R B C 4 75 m cmm   W B C 7,500 per cmm   Polymorphs 67%, lymphocytes 29%, monocytes 4%   Electrocardiographs showed left axis deviation with low voltage T waves in all limb leads   Sodium amytal narcosis reduced the blood pressure from 240/125 to 130/80

On 21st October, 1948, Mr A Dickson Wright removed a large tumour from the region of the pancreas together with the spleen   The tumour weighed 1,100 gms and was apparently encapsulated with adherent pancreas and a piece of normal adrenal gland   Professor W D Newcomb reported the tumour as a phæochromocytoma composed of very varied cells   Some were multinucleated and mitotic figures were frequent   There were numerous large blood spaces   A portion of the tumour was placed in N/10 hydrochloric acid and sent to the Department of Pharmacology at the University of Oxford   Assay (case 2, Holton (30)) showed it to contain the equivalent of 0 3 mg l-adrenaline and 10 mg dl-noradrenaline per gm of tumour tissue

After operation the blood pressure slowly rose from 120/80 on 22nd October to 142/88 on discharge from hospital on 25th November, 1948   Thereafter, she remained well till February, 1949, when the headaches returned   There was no recurrence of the sweating attacks   The blood pressure continued to rise but was extremely labile in the range 220 to 170/110 to 90   When seen on 19th October, 1949, the blood pressure was 230/120 and ankle œdema was present   The fundi showed no exudates or hæmorrhages   A benzodioxane test failed to produce a fall in blood pressure

Case 3   B H , a male, was first seen by Sir John Parkinson when aged 11 years in 1929 (Case 7 of Wolff, Parkinson and White (45))   There was then a history of exhaustion, occasional pallor and a varying pulse rate, often slow   He was always easily tired and for years he had had recurrent attacks in which he was pale and the pulse rate varied between 40 and 65 over a period of a few days   His mother stated that the attacks when he was a child occurred about twice a week, and each lasted 2-3 minutes   He became pale, sat or lay down and then used to return to play   At this distance it is impossible to say whether the attacks were similar to those occurring in adult life   Otherwise he was entirely well and fond of games including football   On examination he was a small child but looked well   The pulse rate was from 50 to 60 and sinus arrhythmia was noted   The blood pressure was 110/75   The heart was of normal size and shape   Electrocardiograms in April, 1929, showed small upright P waves in all leads   The PR interval was well under 0 1 second   The ventricular complexes had the form of left bundle branch block

The present attacks began while serving in the Army in June, 1941   Since then they have continued, varying in severity and frequency, but on the whole getting worse   The frequency varied from one to four a day in good periods to ten to twelve in bad periods   The attacks were always the same and varied only in duration and severity   They began with a sort of weak feeling behind the lower sternum and in the epigastrium, then he felt his heart thumping and the pulse slowed , thus in 1943 his normal pulse was 52 dropping to 36 in a bad attack   He noticed that the pulse not only

slowed but that the beats often came in pairs His face went very pale and his hands, feet, nose and ears were pale and cool In the more severe attacks he noticed a throbbing headache and epigastric pain, felt sick and vomited In the worst attacks the headache was very severe, pins and needles developed in the feet and hands and in two very bad attacks he became short of breath He was unable to micturate during an attack and if an attack came during the act the flow stopped His wife noticed that his face paled before he had any symptoms She might also be awakened at night by an attack because of the noise made by the thumping of his heart beat He only sweated in those attacks that were accompanied by a very intense headache and thought this might be due to the pain He never noticed tremors of the limbs and his mind remained clear A characteristic feature of the attacks was that each one came, then receded and came again, there being perhaps two to ten waves in all , four or five being the usual number During the last year there has rarely been a day without an attack, and before admission he was averaging six a day The attacks were most likely to come on with exertion or opening the bowels, but he knew of nothing which would always precipitate an attack During the three months before admission, and especially during the last six weeks after mumps he always felt jaded and extremely tired between the attacks

He was admitted to St Mary's Hospital on 25th October, 1949, under the care of Dr J W Litchfield who had made a diagnosis of phæochromocytoma The patient was a spare man whose skin and mucous membranes were well coloured Physical examination was entirely negative In particular, the fundi showed normal vessels and no papillœdema The heart was not enlarged No mass was felt in the abdomen and a firm pressure from costal margin to pubes produced no attack The blood pressure ordinarily ran between 120/86 and 136/90 He was seen in 20 attacks lasting from a few to about 15 minutes During them the blood pressure was always above the normal range and was recorded as high as 254/154 The face was always conspicuously pale and this constituted the first sign of an attack Breathlessness was not observed, but he became extremely restless and was greatly disturbed by shivering and rigors In one attack developing in the laboratory the heat elimination from the hand was observed to fall and the face to pale before the patient was aware of the onset The blood pressure both systolic and diastolic rose and the heart rate slowed (Fig 10) The urine occasionally showed a trace of albumen but no deposit

Radiological examination (Dr E Rohan Williams) Chest this revealed no pulmonary lesion The size and shape of the heart and aorta were within normal limits Excretion urography both kidneys showed a normal excretion rate and there was no deflection of the calycine pattern on either side There was, however, a faint suggestion of a rounded soft tissue mass, about 4-5 cm, in diameter, above the upper pole of the right kidney

On 12th November, 1949, Mr A Dickson Wright removed a suprarenal tumour which was situated above the right kidney enveloped in very vascular perinephric fat The tumour weighed 200 gms , was encapsulated and contained a tag of what appeared to be suprarenal cortex on one edge During the operation the pressure rose as high as 255/160 The patient sweated very copiously during the latter part of the manipulation of the tumour and after the suprarenal vein was tied On clamping the suprarenal vein at 10 29 a m the blood pressure was 230/140 The tumour was removed at 10 34 a m , the blood pressure being 160/100 By 10 53 a m the blood pressure had fallen to 115/85 at about which level it subsequently remained
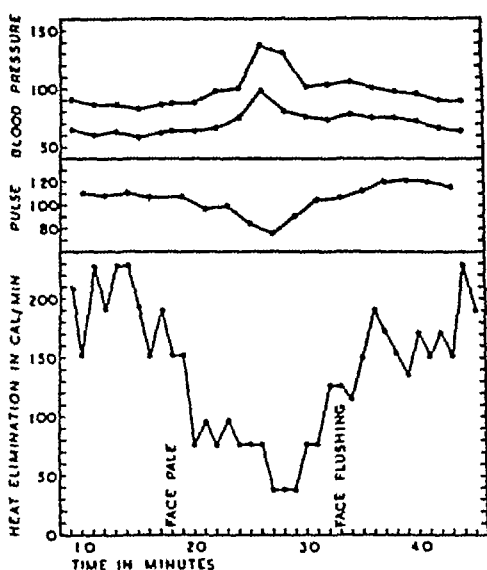
Fig 10   Circulatory changes observed during a paroxysmal attack in Case 3

Pathological examination (Dr R H Heptinstall) showed the tumour to be a phæochromocytoma of the suprarenal gland Histologically it was composed of large polygonal cells, with vesicular nuclei, arranged in diffuse sheets Numerous thin walled vascular channels were present, in addition to numerous empty spaces with no definite lining In bichromate fixed material brownish granules were present in the cytoplasm of the cells A renal biopsy revealed no significant vascular or glomerular changes

When last seen on 22nd December, 1949, he had had no further attacks and felt entirely fit The blood pressure was 130/80

The maximal heat elimination of the hand was determined as in Case 1 with the following results

Date	Maximal heat elimination Cal /min /100 ml hand	Blood pressure
4 11 49	24	130/90
7 11 49	34	95/65
12 11 49	Excision of phæochromocytoma	
23 11 49	72	126/74
22 12 49	76	122/82

A specimen of femoral arterial blood was withdrawn during an attack and heparin was added   The plasma was separated by centrifuging in cooled tubes and was sent packed in ice to Prof J H Gaddum who received it about twelve hours later   Investigation by Dr T B B Crawford failed to detect any significant quantity of noradrenaline or adrenaline   Assay of the tumour is not yet complete but it is known to contain both noradrenaline and adrenaline

Discussion

A consideration of the clinical features in these three cases will show that the majority of the symptoms may be regarded as the effects of an outpouring of adrenaline and noradrenaline from the tumour   In three phæochrome tumours including that of Case 2 of the present series, Holton (30) has demonstrated the presence of both noradrenaline and adrenaline, the former in considerably larger amount   The tumour of Case 3 is also known to contain both noradrenaline and adrenaline   Many of the symptoms may be due to the presence of both substances, but the shivering, restlessness and palpitations are more suggestive of the action of adrenaline   On the other hand, the features of the paroxysmal attack observed in Case 3, particularly the onset of circulatory changes before subjective sensations, the rise in both systolic and diastolic pressures and the cardiac slowing, were all such as were commonly seen in the experimental noradrenaline infusions   This patient stated that with the pronounced cardiac slowing during an attack the beats often came in pairs, a feature previously noted in the experimental infusions of noradrenaline   Similar electrocardiographic changes have been reported during paroxysms of hypertension due to a phæochromocytoma (13, 29)

Little attention has been paid in the past to the disturbances of peripheral blood flow due to the presence of these tumours   Evans and Stewart (18) measured skin and other temperatures in one case and concluded that there was a reduction in total cutaneous blood flow between the attacks , skin blood flow is, however, very variable   In two of our cases the blood flow through the hand was estimated calorimetrically after releasing sympathetic tone by warming the body   This method yields very constant results in a

given normal subject (35)   In Case 3, there was a profound fall in heat elimination during an attack as was expected   What was unsuspected was the reduced heat elimination found in both cases before operation when the patient was symptom free and the blood pressure at its base line (Case 1) or normal (Case 3)   Evidently at that time the vessels of the hand were narrowed by a circulating vasoconstrictor substance and since this constriction disappeared after excision of the tumour it would seem probable that it was due to noradrenaline or adrenaline , more probably the former because adrenaline produces symptoms so much more readily and was found to do so in Case 1   It accordingly seems probable that in these patients there was a continuous release of vasoactive substances from the tumour and that this release was immensely increased during the attacks   Strombeck and Hedberg (42) found in one case an increased blood adrenaline level (their estimation probably included noradrenaline) between the attacks while the blood pressure was normal, and a much higher level during the paroxysms of hypertension

In spite of the evidence obtained from the circulatory observations that noradrenaline was present in the blood stream both between and during attacks, the assay of femoral arterial blood failed to detect its presence in significant amount   Professor Gaddum did not consider the unavoidable delay of twelve hours before the assay was commenced a sufficient explanation for its absence   The presence of vasoconstrictor substances has been demonstrated in the blood of such cases by previous investigators (3, 42) Others, however, have failed to find any (9)   Recently Vogt (44) has demonstrated the presence of noradrenaline in the blood during an attack It should be realised that a quantity of noradrenaline too small for assay by the pharmacological methods at present available will produce profound circulatory disturbances

It has often been assumed that the persistent hypertension seen in some cases of phæochromocytoma before operation is due to the presence of the secretions of the tumour   It is also reported that operation cures the hypertension   Thus, Green (26) in a series of 19 patients with sustained hypertension before operation followed up to three years after removal of the tumour found that in all cases the blood pressure returned to normal In Cases 1 and 2, who both had a persistent hypertension before operation, the blood pressure did not return permanently to normal   In both, the retinal abnormalities slowly disappeared and there were no further paroxysmal attacks   The hand vessels dilated normally after operation   There was thus no evidence for the presence of a second phæochromocytoma and the conclusion that no further active adrenal medullary tissue was present was supported by the negative histamine test in Case 1 and the negative benzodioxane test in Case 2   The latter test was introduced by Goldenberg and others (23) who described a reduction of blood pressure on the administration of F 933, an adrenolytic drug   Prunty and Swan (37)

On the other hand there are some differences between the two states Essential hypertension is not associated with a reduction in forearm blood flow (1) and hand blood flow (35) or bradycardia However, it is not known how long bradycardia would persist in man if noradrenaline were given indefinitely More striking are the changes in facial complexion Many patients with hypertension are highly coloured but with noradrenaline hypertension the face is pale If in essential hypertension, noradrenaline is present in excessive amounts in the circulating blood it must be assumed that the facial vessels do not react to noradrenaline in the same way as in normal subjects We have accordingly given intravenous l-noradrenaline infusions at the rate of 10 μg per minute to hypertensive patients and have shown that they develop facial pallor to the same extent as subjects with normal pressures It has also previously been shown that patients with hypertension pale with the injection of a small quantity of adrenaline (36) Essential hypertension is thus probably not associated with the presence of an excess of free circulating noradrenaline Goldenberg and others have observed that the rise of blood pressure with noradrenaline is greater in subjects with raised than with normal pressures If hypertension were due to an excess of noradrenaline produced and exerting its action locally on the arterioles of the different organs some reduction in sensitivity to injected noradrenaline might be expected

SUMMARY

1 L-noradrenaline when given by intravenous infusion to healthy young adults at a rate of 5 to 30 μg per minute produced few, or no sensations The systolic, diastolic and mean arterial pressures rose, the heart slowed and there was a reduction in the blood flow of muscle and skin, and paling of the skin and mucous membranes

2 Noradrenaline infusion produced a marked fall in the renal clearance of diodone, while the inulin clearance showed only a slight fall or no change the filtration fraction consequently rose These changes are similar to those recorded by other workers following injections of a wide range of pressor drugs and cannot, therefore, be regarded as specific

3 A comparison has been made of the actions of adrenaline and noradrenaline

(a) Their actions on skin, mucous membranes and heat elimination from the hand were similar

(b) At the onset of adrenaline infusions, systolic and diastolic pressures both fell abruptly and there was a severe tachycardia subsequently the blood pressure rose, the systolic exceeding, and the diastolic approximating to normal levels, and the heart rate slowed The forearm blood flow increased

however, have recently shown that it failed to reduce the blood pressure during short term infusions of noradrenaline   Its action might be different in long sustained infusions, but the inferences to be drawn from this test are obviously open to some doubt   In animal experiments it has not been possible to produce a prolonged hypertension by the continuous administration of sympathomimetic amines   With infusions of adrenaline in dogs for several hours, the rise of pressure is not sustained (19) and recent work in this laboratory has shown that it is not possible to produce a persistent hypertension in rabbits with noradrenaline infusions maintained for several days   It should also be noted that cessation of a long infusion of adrenaline in man is followed by a period of considerable hypotension (27, 31)   The mechanism of the development, and the type, of chronic hypertension in these two cases are thus obscure   It is conceivable that the persistently raised blood pressure may be due either to vascular lesions sustained during the paroxysms or to an unassociated essential hypertension   The renal biopsy in Case 1 showed a little hyaline change in some of the afferent arterioles and associated glomerular fibrosis , the majority of the glomeruli were normal   In Case 2, there was no renal biopsy and the only evidence for the presence of vascular lesions was provided by the examination of the optic fundi   The renal function tests were normal in both patients   The absence of arteriolar lesions in Case 3 is remarkable for this patient had been exposed to paroxysms of severe hypertension for many years   Byrom and Dodson (14) produced arteriolar necrosis in animals by sudden short periods of excessive pressure, but in Case 3 such periods of pressure occurred with negative results, at least so far as can be judged from the renal fragment obtained at operation   Essential hypertension is now generally regarded as an inherited condition   In Case 1, apart from a sister who died of eclampsia at 20 years of age, there was no definite family history of hypertension, and an elder brother was found to have a normal blood pressure   The father of the patient in Case 2 was stated to have high blood pressure, but he was alive and active at 74 years   Her mother died of a cerebral thrombosis at 71 years of age   The evidence from the point of view of the family history in these two cases is difficult to assess   It is certainly not possible to exclude an unrelated essential hypertension in both these cases of phæochromocytoma

Essential hypertension has recently been attributed to a disturbance in the proportions of noradrenaline and adrenaline produced at the sympathetic nerve endings, the rise in pressure being due to a relative excess of noradrenaline (22)   There are some similarities in essential and experimental noradrenaline hypertension   In both there is a rise in systolic and diastolic pressure with little subjective disturbance   The renal circulatory changes are also similar, for the characteristic picture of early essential hypertension is one of a lowered diodone clearance, normal inulin clearance and a rise in filtration fraction (24)

(*c*) With noradrenaline the systolic and diastolic pressure rose without a preliminary fall and the heart slowed   The forearm blood flow diminished slightly

4   The bradycardia was thought to be reflexly mediated through the vagus consequent on a rise of systemic arterial pressure for the following reasons —

(*a*) The slowing of the pulse appeared with, or slightly after, the hypertension and in no case preceded it

(*b*) The electrocardiogram showed depression of A-V conductivity of varying degrees

(*c*) In one subject the electrocardiographic changes with noradrenaline were reproduced by carotid sinus pressure

(*d*) The bradycardia was largely abolished by atropine

5   After intravenous atropine sulphate in doses of 1 2 to 2 1 mgm , noradrenaline produced an initial slight bradycardia quickly followed by a considerable tachycardia and a great rise in blood pressure and usually by a rise in forearm blood flow   In one subject venous congestion in the neck was observed   On stopping noradrenaline the tachycardia gradually disappeared though the blood pressure fell promptly

6   Observations on three cases of phæochromocytoma showed that the symptoms and circulatory changes associated with the paroxysmal attacks were such as might be caused by the release of adrenaline and noradrenaline from the tumour   A diminished heat elimination from the hand between the attacks suggested that a vasoconstrictor substance, possibly noradrenaline, was continually present in the circulating blood, even in the absence of hypertension   The chronic hypertension in two cases was not due to the secretions of the tumour and was not relieved by operation

7   Evidence was presented for the view that in essential hypertension, noradrenaline is not present in abnormal amounts in the circulating blood

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EFFECTS OF HEAT AND COLD ON THE DISTRIBUTION OF BLOOD WITHIN THE HUMAN BODY

RADIOLOGICAL INVESTIGATIONS OF THE LIVER, LUNGS AND HEART

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IT is an established fact that the superficial vessels of the body, and particularly those of the extremities, dilate when the environmental temperature rises and constrict when the temperature falls (10, 11)   These changes are associated with variations in the blood flow through the extremities (1) and changes in the amount of blood contained in the limbs (5)   While changes in the blood flow may be compensated by adjustments of the cardiac output (2), an increase or decrease in the amount of blood contained in the surface of the body must be accompanied by variations in the amount of blood contained elsewhere   Indeed, experiments on human subjects have suggested that the lungs contain more blood when the body surface is cool than when it is warm (5, 6) and it was concluded that changes of the environmental temperature caused a redistribution of blood between the superficial vessels and the inside of the body according to the need to preserve or dissipate heat (5)

The present investigation was planned to extend our knowledge of this adaptive mechanism   Experiments on animals have suggested that the amount of blood in the liver can be varied (3, 8, 14) and that the liver may contain vessels which can transfer blood from the arterial to the venous side of the circulation without passing through the capillaries (12)   It has not been shown, however, that the amount of blood in the liver of man can vary and it was decided to find out whether the contours of the radiographic shadow of the liver altered when the environmental temperature changed   Preliminary trials showed that the outline of the liver was clear enough on X-ray films to allow this method to be used   In addition it was decided to study any changes in the vascularity of the lungs and the size of the heart which could be demonstrated by radiological methods

*   *Methods*

Subjects   Seven students aged 20-21 years had volunteered for the experiment   They were not selected, but they all happened to be of slender build   They wore bathing shorts throughout the tests

Procedure   The subjects were tested one at a time, six of them at air temperatures of 20-22° C and one at an air temperature of 25° C   Each subject had a light meal 1 hr   beforehand and then lay on his back on the X-ray table for another hour in order to become adjusted to the horizontal posture   During this period the subjects turned on their faces for a few minutes and preliminary radiographs of the liver, lungs and heart were taken to check the positions and times of exposure   Measurements of the skin temperature, the mouth temperature and the pulse rate were also made in order to accustom the subjects to the experiment   After 1 hr   the subjects turned on their faces again and remained still in that position for about 45 min   while they were cooled and warmed   Three subjects were first warmed and then cooled, while the other four were first cooled and then warmed   They were warmed under an electric cradle and cooled by moist towels and a fan   The heating cradle was placed over the subjects' backs and buttocks, but while radiographs were being taken it was pushed down over their buttocks and thighs   The subjects' feet and toes were exposed throughout the tests   Since the skin over the toes is the last part of the body surface to show reflex vasoconstriction and vasodilatation (11), a fall of the toe temperature to within 1-2° C of the air temperature was considered an adequate degree of cooling, and a rise of the toe temperature to 6-8° C above the air temperature was considered an adequate degree of warming   Under standard conditions such variations undoubtedly indicate significant changes in the amount of blood contained in the surface of the body   Excessive cooling and warming was avoided because it was thought that over-heating of the skin (10) and shivering (6) might alter the distribution of the blood within the body   Nevertheless, subject W was accidentally overheated ,   his mouth temperature did not rise, but his buttocks and thighs were red when the cradle was removed and his toe temperature rose higher than that of any other subject (to 32 5° C , at a room temperature of 20° C)

When warming was complete and again when cooling was achieved, radiographs were taken in triplicate of the liver, the lungs and the heart of every subject   It will be clear from what has been said above that all radiographs were taken with the subjects lying on their faces   In order to make sure that radiographs of the same region were always taken in the same position, the X-ray tube was centered over marks which had been previously made on the subjects' skin   The radiographs of the liver were taken at the end of quiet expiration and those of the lungs and heart at the end of quiet inspiration   The subjects had been trained beforehand to hold their

breath at the instant when they were asked to do so   The times of exposure varied from subject to subject but they were always the same for any one region in any one subject   Intensifying screens were used for the liver and lungs with exposures lasting 1 5-2 sec for the liver and 0 15-0 2 sec for the lungs   Radiographs of the heart were made on Ilfex films without intensifying screens and with exposure times of 3-4 sec , this was long enough to record the borders of the heart at their greatest excursion from the mid-line, and inaccuracies from short exposures which would have shown up the heart in an unknown phase of the cardiac cycle were thus avoided

The skin temperature of the ball of the left big toe was measured with a thermocouple and potentiometer (4), the mouth temperature with a mercury thermometer which was held under the subjects' tongue for 5 min , and the pulse rate by palpating the radial artery for 30 sec

Interpretation of results   F R B , who was not present during the experiments, viewed the radiographs of the lungs of each subject in turn   The films were marked with a code which differed in each subject and of which another of the authors kept the record   F R B graded the vascularity of the lungs in each subject   Since it was impossible to prevent variations in the voltage of the electric mains and in the exposure times, which would have affected the opacity of the films, the vascularity of the lungs was judged from the size and number of the vessels visible in the lung fields and not from the opacity of the radiographs   Thus observations could only be made on the macroscopic pulmonary vessels   The results were subjected to statistical analysis   Tracings of the liver contours and measurements of the transverse diameter of the heart were also made by F R B   For this work also precautions were taken to ensure that F R B did not know which films were taken after cooling or after warming

RESULTS

Fig 1 gives the outlines of the liver of each subject at the end of cooling and warming   Because the whole of the liver contour could not be completed on the radiographs the areas of the liver shadows were not measured, but all the tracings shown in Fig 1 were reduced to the same scale which is given in centimetres   In one subject (J) the difference in the liver contours allowed no conclusions, but in the other six subjects the liver appeared larger when the body surface was cool than when it was warm   There were no appreciable variations in the contour of the liver in any batch of three films taken of the same subject at the same time   The height of the diaphragm was not always the same when the subjects were cold as when they were warm (Fig 1)   In order to find out whether changes in the depth of respiration could influence the size of the liver shadows, several radiographs of the liver of another subject were taken at constant environmental

temperature during different phases of respiration   It was found that such variations in the height of the diaphragm as those observed in the present experiment did not alter the outlines of the liver
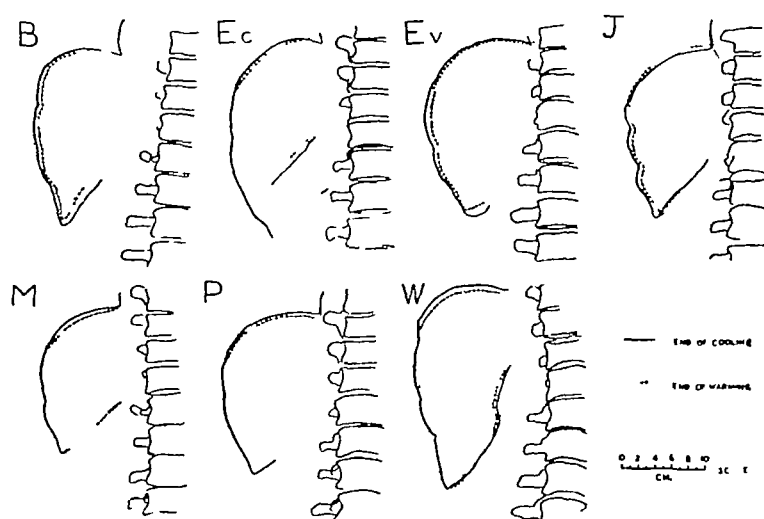
Fig 1   Tracings of the radiographic contours of the liver at the end of cooling (continuous line) and at the end of warming (dotted line)

Fig 2 gives a typical example of the appearance of the lungs   When F R B 's gradings were compared with the records (Table I) it was found that 32 out of 42 radiographs were graded in accordance with the hypothesis that the lungs held more blood in a cool environment than a warm one The only discrepancies were as follows   (1) No opinion was given on 2 films of J 's lungs   (2) Of the 3 radiographs of P 's lungs which were considered to show the greatest degree of vascularity one had been taken when that subject was warm   (3) All 3 radiographs of W 's lungs which were considered to show the greatest degree of vascularity were taken when that subject was warm   Each batch of 6 radiographs showing the lungs of one subject was statistically treated as a $2 \times 2r$ correlation between the experimental conditions and F R B 's grading (15)   The correlations for each subject were summated and tested against the summated variance, which gave for the whole group of 42 films the normal deviate of 3 43 (p = 0 001)   The correlation, therefore, was statistically significant

The transverse diameter of the heart shadows never varied by more than 2 mm (or 1 3%) in any of the six radiographs taken of any one subject The mouth temperature never varied by more than 0 2° C in any one test The pulse rate of all subjects was 6-24 beats/min   (10-24%) higher when their body surface was warm than when it was cool

TABLE I

Radiologist's grading of the X rays of the lungs and the statistical analysis

(*See text for experimental plan*)

Subject	3 X rays of the lungs taken at the end of cooling	3 X rays of the lungs taken at the end of warming	Difference numerator for Kendall r 2 × 2 case S	Correlation coefficient r	Variance of S
B	G V G V G V	L V L V L V	+ 9	+ 1 000	16 2
Ec	G V G V G V	L V L V L V	+ 9	+ 1 000	16 2
Ev	G V G V G V	L V L V L V	+ 9	+ 1·000	16 2
J	G V G V No grading given	L V L V	+ 8	+ 0 770	19 2
M	G V G V G V	L V L V L V	+ 9	+ 1 000	16 2
P	G V G V L V	L V L V G V	+ 3	+ 0 333	16 2
W	L V L V L V	G V G V G V	— 9	— 1 000	16 2

G V denotes radiologist's grading of greater vascularity of the lungs

L V denotes radiologist's grading of lesser vascularity of the lungs

ΣS = 38

Mean r = + 0 59

Σ var S = 116 4

$$\text{Normal Deviate} = \frac{38 - 1}{\sqrt{116\ 4}} = 3\ 43$$

DISCUSSION

The present investigation shows that radiological studies can give useful information about physiological processes if care is taken to use a satisfactory technique   It is unlikely that rapid changes in the size of the liver shadow could be caused by anything other than changes in the amount of blood in that organ   It seems proper, therefore, to conclude that the blood content of the human liver can vary   Direct radiological evidence moreover, has given further support to the view that the amount of blood in the lungs can also vary (7) and that more blood is contained in them in a cool environment than in a warm one (5, 6)   Pulmonary congestion in heart failure is not confined to the microscopic blood spaces and it has been

suggested that vessels other than the alveolar capillaries probably participate in the function of storing blood (5)　The present investigation also suggests that the larger vessels of the lungs may open up and close down under physiological conditions　Subject W , who was the only one to show an increase in the vascularity of his lung fields when he was warm, had been exposed to excessive radiant heat, and his superficial vessels may have contracted as a protective reflex to a strong heat stimulus (10)　The fact that the liver of this subject became smaller (Fig 1) when the vascularity of his lungs increased suggests that the amount of blood in these two organs may be controlled by different mechanisms　The findings that the pulse rate was invariably higher when the subjects were warm than when they were cool while the transverse diameter of the heart remained unchanged suggests that the cardiac output may have varied as a result of changes in the heart rate, and this conforms with previous observations (2, 9, 13)

Although there is no definite evidence that the human body contains any sack-like storage vessels which can withdraw blood from the circulation, except perhaps the spleen, it seems probable that the amount of blood in all highly vascular regions can vary to suit the needs of other parts of the body It remains to be seen whether the liver and lungs are specifically adapted to such a function

SUMMARY

1　In seven normal young subjects radiographs of the chest and abdomen were taken under standard conditions during exposure to cold and warm environments

2　The contour of the liver, as estimated radiologically, is larger and the radiographic shadows of the pulmonary vessels are wider and more numerous when the skin is cooled than when it is warmed

3　These findings suggest that the lungs and liver contain more blood when the skin is exposed to cool than to warm environments

4　The transverse diameter of the heart remained constant in recumbent men following changes of the environmental temperature

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A B

Fig 2   The same area of the left lung A at the end of cooling B at the end of warming Subject B   (These radiographs should be judged on the size of the vascular shadows and not on opacity)

THE CIRCULATORY EFFECTS OF HIGH SPINAL ANÆSTHESIA IN HYPERTENSIVE AND CONTROL SUBJECTS

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DURING high spinal anæsthesia the blood-pressure, although somewhat reduced, does not as a rule fall sufficiently low to produce symptoms of collapse Smith and others (19) were the first to suggest that such fall in blood-pressure as occurs is due to a reduction in cardiac output Rovenstine and his colleagues (15), who determined cardiac output by the ballistocardiographic method, obtained results which supported this suggestion It was further demonstrated that the total effective peripheral resistance, expressed as the ratio of mean blood-pressure to cardiac output, showed little deviation from pre-anæsthetic levels These studies were conducted on normal subjects, lying supine in the horizontal plane, and without the complication of surgical intervention These workers concluded, therefore, that the blood-pressure in normal subjects in the supine position is less dependent on tonic activity of the autonomic vaso-constrictor nerves than has been commonly supposed

In patients with hypertension the fall in blood-pressure during spinal anæsthesia is greater than in normal subjects Allen and others (1) found that spinal anæsthesia lowered the blood pressure to approximately normal levels in all their hypertensive patients Lumbo-dorsal sympathectomy, on the other hand, produces a less constant reduction of blood-pressure in hypertensives than does spinal anæsthesia , hence spinal anæsthesia has been abandoned as a method of quantitative prediction of the results of sympathectomy

The work reported here was undertaken to find out whether the larger reduction in blood-pressure in hypertensive patients is due to the fact that they have a greater degree of autonomic vaso-constrictor tonus than in normal subjects, or whether the difference can be accounted for by changes in cardiac output

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Circulatory effects of spinal anæsthesia

In the first column below case number, sex and age, are recorded the upper levels of anæsthesia to pinprick and the time in minutes from the full development of anæsthesia to end of the second tilt All figures in the table are means of at least two observations

Case No	Sex, Age			Mean B P (mms Hg)	Cardiac output (litres/min)	Right auricular pressure (cm saline) Sternal angle = 0	Heart rate (rate/min)	Total peripheral resistance (BP/CO)	Duration of tilting under Sp An (minutes)
1	M 48 Th 6 0 30 mns	Before anæsthesia, subject ,, ,, During ,,	supine tilted supine tilted	100(120/80) 103(120/85) 86(105/65) 48(65/30)	3 7 3 1 2 6 2 2	— 4 — 8 — 5 —10	70 72 74 50	27 0 33 2 32 7 21 2	7
2	F 65 Th 5 10 mins	Before ,, During ,,	supine tilted supine tilted	105(130/80) 108(135/80) 38(50/25) —	6 7 5 7 3 0 —	— 2 — 4 — 6 —	75 78 69 —	15 65 19 0 10 6 —	7
3	M 61 Th 4 32 mns	Before ,, During ,,	supine tilted supine tilted	115(155/75) — 95(125/65) 61(74/48)	4 3 3 5 3 7 2 6	— 4 — 5 — 6 0 5	75 72 72 60	26 7 — 25 7 23 5	7
4	F 54 Th 5 32 mins	Before ,, During ,,	supine tilted supine tilted	121(145/97) 117(135/100) 105(125/85) 90(112/80)	5 6 5 2 4 0 3 9	— 6 —10 — 7 —12	60 76 76 120	21 7 22 5 22 8 24 0	8
5	F 54 Th 5 6 45 mins	Before ,, During ,,	supine tilted supine tilted	143(190/96) 140(190/90) 109(135/83) 75(90/60)	3 2 2 8 2 7 1 8	— 3 5 — 0 0 — 6 0 — 7 5	82 75 86 46	44 75 48 3 40 5 41 6	2
6	M 40 Th 5 30 mins	Before ,, During ,,	supine tilted supine tilted	148(185/110) 144(178/110) 118(135/100) 82(95/68)	8 5 5 0 5 0 3 8	— 2 — 4 — 6 — 7 5	104 104 110 72	17 4 28 8 23 6 21 0	10

										7
7 M 20 Th 2 32 mins	Before	„	„	supine	160(180/120)	5 6	—	82	20 8	
	„	„	„	tilted	—	—	—4	—	—	
	During	„	„	supine	05(120/70)	5 3	—4	76	17 0	
	„	„	„	tilted	77(187/90)	3 9	—7	60	10 7	
										21
8 M 35 Th 4 15 mins	Before	„	„	supine	103(103/112)	8 1	—5	70	20 1	
	„	„	„	tilted	104(105/173)	9 1	—10	74	20 2	
	During	„	„	supine	142(170/111)	7 0	—7	72	20 3	
	„	„	„	tilted	115(130/100)	6 1	—12	80	18 5	
										14
9 F 32 Th 1½ 15 mins	Before	„	„	supine	105(200/130)	8 0	—2	97	18 5	
	„	„	„	tilted	—	—	—	—	—	
	During	„	„	supine	100(120/80)	0 0	5 6	83	10 0	
	„	„	„	tilted	98(113/83)	0 5	6 6	92	15 0	
										9
10 F 62 Th ½ 15 mins	Before	„	„	supine	172(225/120)	5 6	—5 5	94	30 7	
	„	„	„	tilted	—	—	0 5	71	—	
	During	„	„	supine	137(170/104)	4 5	0 5	80	30 5	
	„	„	„	tilted	110(148/84)	3 8	—	—	30 6	
										2
11 M 53 Th 1 20 mins	Before	„	„	supine	173(220/125)	1 0	—5	80	37 0	
	„	„	„	tilted	170(210/170)	4 1	—7	94	41 5	
	During	„	„	supine	105(120/00)	3 0	—7	72	35 0	
	„	„	„	tilted	50(80/20)	2 7	—10	61	18 5	
										10
12 M 41 Th 1 5 12 mins	Before	„	„	supine	175(210/110)	1 3	—4	88	40 7	
	„	„	„	tilted	155(180/130)	3 0	—7	84	30 6	
	During	„	„	supine	110(105/115)	3 7	—6	80	37 9	
	„	„	„	tilted	15(60/30)	2 7	—10	60	10 7	
										8
13 M 13 Th 1 5 50 mins	Before	„	„	supine	180(230/180)	4 3	—1	82	42 0	
	„	„	„	tilted	—	—	—3	—	—	
	During	„	„	supine	137(170/104)	3 0	5 6	75	38 0	
	„	„	„	tilted	139(108/110)	3 0	5 6	70	38 6	
										5
14 F 57 Th ½ 20 mins	Before	„	„	supine	105(200/130)	5 5	0	90	35 5	
	„	„	„	tilted	181(232/130)	4 0	0	90	30 5	
	During	„	„	supine	130(155/105)	4 4	—7	44	20 5	
	„	„	„	tilted	90(115/66)	3 6	—7	34	25 7	

Clinical material and methods

Fourteen subjects were studied, nine had a severe degree of essential hypertension with diastolic pressures of 110 mm Hg or over, and five control subjects had normal or slightly elevated blood-pressures, with diastolic levels not exceeding 100 mm Hg In the patients with normal blood-pressures spinal anæsthesia was induced for small operations These were not undertaken until the experimental observations had been completed

Spinal anæsthesia was induced, without premedication, by the intrathecal injection of light nupercaine Anæsthesia to pinprick extended to a level between the fourth and sixth, and, in one case, to the second thoracic segments In every case warming and flushing of the toes was observed

Serial measurements of blood-pressure, heart rate, cardiac output and right auricular pressure were made before and during spinal anæsthesia Right auricular pressure and cardiac output were measured by the method of cardiac catheterisation (11) The right auricular pressure was recorded on a saline manometer and related to the level of the sternal angle which was taken as zero The oxygen unsaturation of 3 cc samples of right auricular and arterial blood was determined by the Haldane method (6) Oxygen consumption was measured by spirometry Arterial blood-pressure was measured by the auscultatory method, a four-inch cuff being used True mean blood-pressure values based on planimetric measurement of the area of pulse-pressure curves were not obtained, but changes in blood-pressure were followed by taking the averages of systolic and diastolic figures, these, for convenience, are referred to as " mean B P " Changes in total peripheral resistance (T P R) were followed by calculating values in arbitrary units from the formula total peripheral resistance = blood pressure/cardiac output (2) For this purpose the average of two or more values of mean blood pressure and the average of duplicate values of cardiac output were employed

Experimental routine

The experiments began with the passage of the cardiac catheter Serial observations were started when the pulse-rate had returned to its resting level, usually after half an hour, the subjects remaining quietly at rest in the supine position Oxygen consumption was measured at this stage and in six cases again during spinal anæsthesia

After a satisfactory base line had been obtained, some of the subjects were tilted feet downwards and were kept in this position until blood-pressure and heart rate had become constant The tilting procedure consisted of raising the head of the bed or tilting the operating table until the subject was inclined at an angle of 30° to 40° to the horizontal plane

The bed or table was then replaced in the horizontal position and spinal anæsthesia was induced Circulatory measurements were continued as soon

as the anæsthetic had become fully effective, as shown by the level of response to pinprick and by flushing of the toes   When heart rate and blood-pressure had assumed constant levels, the patients were again tilted feet down   If, as usually happened, symptoms of incipient collapse appeared, the subjects were quickly returned to the horizontal position and given an injection of the sympatheticomimetic amine, veritol, which restored the blood-pressure to pre-anæsthetic level

Assessment of the accuracy of results

In order to establish the validity of results obtained by methods of measurement for which no high degree of precision can be claimed, estimates of the influence of random or uncontrolled variation were made   The sources of random variation are errors inherent in the methods of measurement and spontaneous physiological fluctuations independent of alteration in the experimental conditions   The estimates were based on 40 to 60 values of each variate, obtained when the subjects were judged to have reached a steady state during the several stages of the experiment   Sets of pairs or multiple values from all subjects were analysed and estimates of variance calculated   Values for the standard error of the mean of duplicate measurements were as follows   blood-pressure $\pm$ 2 5 mm Hg , arterio-venous oxygen differences $\pm$ 1 72 (2 4%), heart rate $\pm$ 3 beats per minute, right auricular pressure $\pm$ 1 cm saline   In the cardiac catheterisation technique, measurements of cardiac output by the Fick principle are based on the measured arterio-venous oxygen differences and a single value of oxygen consumption, on the assumption that the latter is fairly constant under resting conditions   The use of a single value for oxygen consumption in calculating a series of cardiac outputs introduces a possible source of variation which does not appear in the cardiac output figures   Further work is needed to assess the importance of this source of error   In the present experiments there was no evidence of a systematic fall in oxygen consumption due to the anæsthesia   The coefficient of variation of individual values of oxygen consumption, derived from the six available pairs of measurements was 6 13%   The cardiac output being measured as the ratio of oxygen consumption to arterio-venous oxygen difference, its coefficient of variation may be obtained from the approximate

formula $\left\{ \begin{matrix} \text{coefficient of variation} \\ \text{of cardiac output} \end{matrix} \right\}^2 =$

$\left\{ \begin{matrix} \text{coefficient of variation} \\ \text{of } O_2 \text{ consumption} \end{matrix} \right\}^2 + \left\{ \begin{matrix} \text{coefficient of variation} \\ \text{of A-V } O_2 \text{ difference} \end{matrix} \right\}^2$ (4) (20)

In the present case assuming the two measurements to be independent, this formula gives a coefficient of variation of cardiac output of 6 6%, so that differences of 13% may be considered suggestive of a real change   In the same way, the coefficient of variation of T P R is found to be of the order of 7%, so that differences of over 14% may be considered significant of a real effect

TABLE II

Changes in blood pressure, cardiac output, and total peripheral resistance before and during spinal anæsthesia, expressed as percentage deviation from control values before anæsthesia

Case No	Blood pressure			Cardiac output			Peripheral resistance				Comment
	Tilted before Sp An A	Horizontal during Sp An B	Tilted during Sp An C	Tilted before Sp An A	Horizontal during Sp An B	Tilted during Sp An C	Tilted before Sp An A	Horizontal during Sp An B	Tilted during Sp An C	Change in T P R due to tilting during Sp An C–B	
1	+2·5	−15·0	−52·5	−10·2	−20·7	−40·5	+23·0	+21·1	−19·3	−40·4	Immediate fall in T P R on tilting during spinal anæsthesia
2	+2·4	−64·0	—	−15·0	−40·2	—	+21·5	−35·4	—	—	Large fall in T.P.R. during spinal anæsthesia without tilting Circulatory collapse
3	—	−17·4	−45·5	−18·6	−14·0	−39·5	—	−3·8	−12·0	−8·2	
4	−4·0	−14·0	−22·5	−7·2	−17·7	−48·2	+3·7	+5·1	+13·4	+8·3	
5	−2·1	−23·8	−47·5	−12·5	−15·6	−43·6	+7·9	−9·5	−7·0	−2·5	Pallor, faintness, nausea on tilting during spinal anæsthesia
6	−2·1	−20·5	−44·8	−41·2	−41·2	−54·1	+65·0	+35·6	+24·0	−11·6	Values of T P R influenced by resting C O (Table III) Subject nervous
7	—	−33·0	−48·0	−5·4	−5·4	−30·1	—	−33·1	−26·5	+6·6	

SPINAL ANÆSTHESIA IN HYPERTENSION

No.								Sudden fall in T.P.R. after 8 minutes tilted during spinal anæsthesia	Immediate fall in T.P.R. on tilting during spinal anæsthesia	Slow fall in B.P. during 20 mins in tilted position under spinal anæsthesia
8	+0·0	-12·6	-20·2	0·0	-13·6	-24·7	+1·0	+1·0	-5·9	-6·9
9	—	-30·5	-40·6	—	-32·5	-27·0	—	-10·3	-18·4	-8·1
10	—	-20·4	-32·5	—	-19·6	-32·1	—	-0·6	-0·6	0
11	-1·4	-30·5	-70·4	-6·0	-34·8	-41·4	+10·4	-6·9	-51·0	-44·1
12	-11·4	-20·0	-74·0	-9·3	-14·0	-37·2	-2·7	-6·9	-59·0	-52·1
13	-24·0	-24·5	—	-16·3	-16·3	—	—	-8·1	+1·4	—
14	-33·3	-54·0	-16·3	-20·0	-30·4	+11·3	-16·9	-27·6	-11·7	-7·2

The circulatory responses of hypertensive and control subjects were essentially similar, they are therefore considered together   The results are summarised in Tables I and II and typical results are illustrated diagrammatically in Fig 1

1   *Effect of tilting before anæsthesia*

Eight of the nine subjects tilted before anæsthesia showed a rise in T P R, but the rise, with three exceptions, was below the level required for significance   It thus became clear that the method of demonstrating sympathetic paralysis by comparing changes in T P R on tilting before anæsthesia with changes on tilting during anæsthesia could not yield significant results in individual cases, possibly because the angle of tilting was too small   In all fourteen cases, however, warming and flushing of the toes was observed, and muscular paralysis of the abdominal wall and the lower limbs showed that the concentration of local anæsthetic was more than sufficient to paralyse the sympathetic nerve fibres leaving the cord below the segmental level of anæsthesia (16)   On these grounds it was assumed that sympathetic paralysis occurred in all cases

2   *Effect of anæsthesia alone*

During anæsthesia in the horizontal position both blood-pressure and cardiac output fell   In six hypertensive and three control subjects the fall in blood-pressure was approximately proportional to the change in cardiac output, and there was no significant change in T P R   In two of these subjects (Cases 9 and 11) the blood-pressure fell from 200/130 and 220/125 mm Hg to 120/80 and 120/90 mm Hg respectively, showing that in some hypertensive subjects under spinal anæsthesia a fall in blood-pressure to normal levels may be the result of a fall in cardiac output alone

A significant fall in T P R suggesting the presence of an unusual degree of sympathetic vasoconstrictor tonus before anæsthesia was found in two of eight hypertensive subjects and one of five controls   In Case 7, blood-pressure fell from 180/120 to 120/70 mm Hg as result of a 33% fall in T P R and a negligible fall in cardiac output, in Case 14, blood-pressure fell from 260/130 to 155/105 mm Hg as result of a fall in T P R of 16 9% and a fall in cardiac output of 20 0%   In the control subject (Case 2) blood-pressure fell from 135/80 to 50/25 mm Hg and symptoms of collapse developed, though without bradycardia   There was a 35 4% reduction of T P R and cardiac output fell from 5 7 to 3 6 L per minute, a change of 46 2%

The data on two cases (Numbers 1 and 6) have not been used in assessing the effect of spinal anæsthesia on T P R, because T P R, after a large rise on tilting, failed to resume its former level before induction of spinal

anæsthesia, with the result that the level during anæsthesia remained considerably higher than the control levels In other respects the findings in these two cases are comparable with the rest
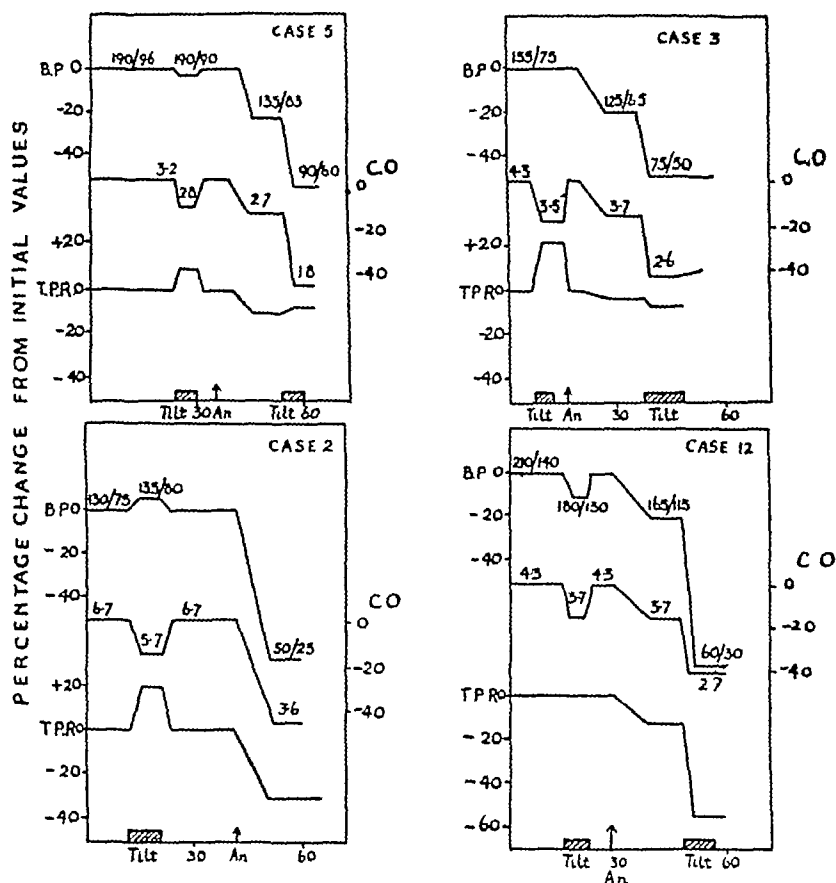

Fig 1 Circulatory responses to spinal anæsthesia and tilting Ordinate percentage changes in mean blood pressure (B P), cardiac output (C O), and total effective peripheral resistance (T.P.R) Abscissa time in minutes

Note the following

(1) Effect of tilting before anæsthesia minimal change in B.P fall in C O compensated by rise in T P.R   (2) Effect of anæsthesia Cases 3 5 and 12 show the typical response , moderate fall in B P with corresponding reduction in C O minimal change in T P R (3) Effect of tilting during anæsthesia further fall in B.P due to fall in C O , T.P R unchanged Cases 3 and 5   (4) Circulatory collapse induced by anæsthetic alone in Case 2 and by tilting during anæsthesia in Case 12 profound fall in B.P with large fall in C O and T.P.R

Comparison of the magnitude of the blood-pressure changes in the two groups indicated that the fall in blood-pressure in hypertensive subjects, though larger in absolute figures, was not greater than in control subjects when expressed in terms of percentage deviation from the initial level (Table III)

TABLE III

Percentage fall in B P induced by spinal anæsthesia

	No of cases	B P Fall	
		0—25%	Greater than 25%
Normals, or slightly raised B P	5	3	2
Hypertensive subjects	9	5	4

This finding is to be expected because the fall in blood-pressure was mainly due to reduction in cardiac output and the cardiac output changes were comparable in the two groups   For example if T P R remains constant, it follows from the formula, T P R $= B P /C O$, that a 50% fall in cardiac output will be associated with a 50% fall in blood pressure

3   *Effect of tilting under anæsthesia*

Tilting the subject during anæsthesia produced a further reduction in cardiac output and this was associated with a further fall in blood-pressure (Table II , and in cases 3 and 5, in Fig 1)   Comparison of the values of T P R during tilting with those in the horizontal position (Table II, last column) showed no further significant reduction in T P R in ten of thirteen cases   In these ten cases the fall in blood-pressure could be accounted for by the further reduction in cardiac output   In three other subjects there occurred, in addition to the fall in cardiac output, a large fall in T P R , and blood-pressure fell to a very low level   This reaction occurred suddenly, within a minute or two of tilting in Cases 1 and 11, and in Case 12 after an interval of eight minutes during which blood-pressures fell slowly   In the presence of vasomotor paralysis below the level of anæsthesia such a fall of T P R cannot be accounted for by a further release of vasoconstrictor tone in the anæsthetised region of the body   Vasodilatation above the level of anæsthesia must, therefore, have occurred

Most subjects showing large falls in blood-pressure developed symptoms such as faintness, giddiness and nausea, accompanied by pallor, and, in some cases, sweating and changes of respiratory rhythm   Such symptoms were more severe in the cases with a fall in T P R   than in other subjects

4   *Changes in heart rate and right auricular pressure*

Changes in heart rate were a feature of the circulatory response to spinal anæsthesia and to changes of body position   Cardiac slowing occurred in association with the larger falls in blood-pressure , but with reduction in blood-pressure of less than 25%, either no change or an increase in heart rate was observed   No correlation was established between reduction in T P R and cardiac slowing , but an increase in T P R on tilting the body before spinal anæsthesia was associated with cardiac acceleration

A fall in right auricular pressure accompanied reduction in cardiac output in most instances, but was not always proportional to it , the coefficient of correlation (r) was 0 65   Further statistical analysis suggested that the association between right auricular pressure and cardiac output was disturbed by the effect of cardiac slowing on cardiac output   Thus, the coefficient of partial correlation of right auricular pressure and cardiac output with heart rate eliminated was 0 9

Discussion

The main factor determining the fall in blood-pressure in hypertensive subjects under spinal anæsthesia appears to be a fall in cardiac output   By this alone, blood-pressure may be reduced to a normal level   In only a quarter of our hypertensive cases was there evidence of a fall in total peripheral resistance greater than fourteen per cent   These findings explain the failure of spinal anæsthesia to predict the results of sympathectomy

Richards (14) has described circulatory studies by Cournand's group on five hypertensive patients after sympathectomy   The average figures for cardiac output before and after operation were 5 70 and 5 52 litres/minute respectively, and there was a reduction in total peripheral resistance of 13%   These findings differ significantly from the effects of spinal anæsthesia in our hypertensive subjects in whom the mean reduction in cardiac output was from 6 2 to 4 7 litres/minute and the reduction in total peripheral resistance 5 4%   The fall in mean blood-pressure was 15 6% in Cournand's series compared with 26 5% in our cases   The relatively large fall in blood-pressure during spinal anæsthesia, compared with that observed after sympathectomy in hypertensive patients, is thus accounted for by the larger reduction in cardiac output by spinal anæsthesia

During these experiments on spinal anæsthesia the upper part of the body remained unanæsthetised   The possible effect of compensatory vasoconstriction must therefore be considered   Vasoconstriction in the fingers and vasodilatation in the toes at the onset of spinal anæsthesia have been demonstrated by simultaneous plethysmography , and it has been inferred that finger vasoconstriction is an index of vasoconstriction in a larger, but not exactly defined, vascular bed of the upper portion of the body (5, 13)   Midwilsky and de Vries (12) claim to have shown that

compensatory vasoconstriction in the upper extremities is the mechanism preventing a large fall of blood-pressure in spinal anæsthesia   They observed hypotension only in cases with an upper level of sensory anæsthesia at or above the fourth thoracic segment   Anæsthesia to this level was associated with a rise in finger temperature which they attributed to paralysis of the sympathetic innervation of the upper limbs   Finger temperatures were measured in four of our cases, a rise being recorded in two cases with upper levels of anæsthesia at Th 3-4 and Th 2 respectively, and not in two others with upper levels at Th 4-5   None of these cases developed hypotension under spinal anæsthesia alone   Calculation of the possible effect of upper limb vasoconstriction on total peripheral resistance renders Midwilsky and de Vries' interpretation improbable   The volume of the upper limbs, measured in two 75 Kg subjects by the method of displacement, was found to be approximately 650 cc   At a blood flow of 3 cc per 100 cc of tissue per minute (2, 3) the total blood flow to the upper limbs would be of the order of 200 cc per minute   On the principle that total peripheral resistance is composed of a number of separate resistances in parallel, the proportion of total peripheral resistance contributed by a region of the body with a blood flow of less than 20% of the total systemic blood flow is necessarily small   Compensatory vasoconstriction, to be effective in maintaining blood pressure, must involve a region with a large blood supply, such as the brain   But Kety and his associates (10), in a recent report on the cerebral circulation in hypertensive subjects under differential spinal sympathetic block, found that, although cerebral blood flow fell, the vascular resistance of the brain did not increase   As the brain is the only organ above the level of anæsthesia known to have a large blood flow and as cerebral vasoconstriction has been shown not to occur, the effect of compensatory vasoconstriction in preventing a fall of blood pressure under spinal anæsthesia is probably of little significance

The state of collapse observed in some subjects tilted under spinal anæsthesia was indistinguishable from a fainting attack   Fainting attacks occur in many conditions in which the output of the heart is reduced, and are associated with a fall of total peripheral resistance (2)   Such a fall in total peripheral resistance was observed in three cases tilted under anæsthesia, but in another case (Case 5) a similar clinical picture including faintness, dizziness, pallor and bradycardia was associated with a very low cardiac output and no change in total peripheral resistance , evidently the mechanism underlying this type of circulatory collapse cannot with certainty be inferred from the clinical picture alone

A fall in total peripheral resistance in subjects tilted under anæsthesia was regarded as evidence of vasodilatation in the unanæsthetised parts of the body   Confirmation of this view has since been obtained by forearm plethysmography (17)   The fall in peripheral resistance in other types of fainting has been considered adequately explained by reflex muscle vaso-dilatation (2, 3)   In the present instance, however, the quantitative effect

of vasodilatation in the muscles with intact sympathetic innervation was not sufficient to account for the whole of the fall in total peripheral resistance It seems likely that vasodilatation may occur in the brain in addition to the muscles Scheinberg and Stead (18) found a 15% reduction in cerebrovascular resistance with a 21% fall in cerebral blood flow in subjects tilted upright, Kety and Schmidt (8) found a 33% fall in cerebrovascular resistance in subjects inhaling 7% CO_2, and 10% oxygen respectively, and a similar reduction was found in patients in diabetic coma (9)

The following calculation shows the probable contribution to total peripheral resistance of a fall in vascular resistance both in the muscles and in the brain —

Let it be assumed that the muscles with intact sympathetic innervation comprise one quarter of the total muscle mass and that their blood supply is about 200 cc per min —one quarter of a total muscle blood flow of 800 cc per min (2) Kety and Schmidt's mean figure for cerebral blood flow is 54 cc per 100 g of brain per min (7) The total cerebral flow for a 1400 g brain is therefore about 750 cc per min Let it be assumed that on tilting under spinal anæsthesia cerebral vascular resistance falls by 33%, and that the blood flow in the muscles with intact sympathetic innervation increases two and a half fold, as in other types of fainting (2, 3) From the formula, resistance = blood pressure/blood flow, the approximate values for total peripheral resistance, and regional resistance before and during tilting under anæsthesia have been computed as follows —

CASE 11

SPINAL ANÆSTHESIA	Total peripheral resistance	Resistance in muscles above sp an	Resistance in brain	Resistance in rest of body
1 *Before tilting* B.P 105 mm.Hg C O 3 0 L/min B F muscles above sp an 200 cc /min	$\dfrac{105}{3\ 0} = 35$	$\dfrac{105}{0\ 20} = 545$	$\dfrac{105}{0\ 25} = 140$	$\dfrac{105}{2\ 05} = 51$
2 *During tilting* B.P 50 mm.Hg C O 2 7 L /min B.F muscles above sp an 500 cc /min.	$\dfrac{50}{2\ 70} = 18\ 5$	$\dfrac{50}{0\ 50} = 100$	$\dfrac{50}{0\ 54} = 93$	$\dfrac{50}{1\ 66} = 30$

The percentage fall in resistance of the rest of the body =

$$\frac{51-30}{51} \times 100 = 43\%$$

Calculated in the same manner, the fall in the resistance in the rest of the body during tilting was nil in Case 1 and 56% in Case 12 In two of the three cases, therefore, the fall in total peripheral resistance on tilting under anæsthesia is not completely accounted for by vasodilatation in the muscles of the upper part of the body and in the brain

The mechanism of the fall in cardiac output which played a dominant part in determining blood-pressure changes will not be discussed in detail It is pointed out that the reduction in cardiac output was found to be closely related to the fall in venous filling pressure   There is no evidence that a decrease in intramuscular pressure occurs under spinal anæsthesia (12) Hence the fall in cardiac output may be secondary to relaxation of venous tone as result of sympathetic paralysis   The corollary of this would be that the role of the sympathetic nervous system in determining the resting level of arterial blood-pressure in the horizontal position depends ultimately on control of the venous side of the circulation at least as much as on control of the arterioles

Summary

1   The circulatory effects of spinal anæsthesia carried to the level of the fourth to sixth thoracic segment have been studied in nine hypertensive and five control subjects with normal pressures by the method of cardiac catheterisation

2   A fall in blood-pressure occurred in all cases   This was accounted for mainly by a fall in cardiac output   Although total peripheral resistance showed a tendency to fall, the changes were too small to be demonstrated convincingly in individual cases by the methods used

3   In three out of eight hypertensive cases the blood pressure fell to normal levels   In two of the cases this could be explained by reduction of cardiac output alone ,   in the third, cardiac output and total peripheral resistance were equally involved

4   Although the fall in blood-pressure was greater in hypertensive than in normal subjects, the percentage change from resting levels was comparable in both groups

5   Evidence of an unusual degree of resting sympathetic vasoconstrictor tone was obtained in two out of eight hypertensive cases and one of five controls

6   States of acute hypotension resulting from tilting the body during spinal anæsthesia were studied   Evidence is presented that in some cases a fainting attack occurred, with vasodilatation above the level of anæsthesia

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REGULATION OF SODIUM EXCRETION IN NORMAL AND SALT-DEPLETED SUBJECTS

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THE osmotic stability of body fluid depends on control of the serum sodium within a fairly narrow range of some 137-147 mEq /l   The efficiency of this control of serum sodium is a commonplace of physiology, but the mechanisms involved are not completely understood   It is known that wide variations in dietary intake of sodium are associated with only trivial changes in the serum sodium level, so that renal excretion of sodium effectively compensates for changes in the sodium intake, and in the amount of sodium lost by sweating   In reviewing the excretion of sodium, Wesson, Anslow and Smith (22) explain diurnal variations in sodium excretion in terms of small variations in glomerular filtration rate (GFR) and serum sodium   Such variations produce changes in the sodium load presented for excretion which are small in relation to the total sodium load, but large in relation to the amount of sodium ultimately excreted in the urine   For example, a 1% change in GFR from 100 to 101 ml /min at a serum sodium level of 140 mEq /l adds 0 14 mEq of sodium to the sodium load , this is as much as is commonly excreted per minute in the final urine, so that if no change took place in the amount reabsorbed the sodium excretion might be doubled by a very small change in GFR, such as could not be measured or even detected with certainty   Such a concept presents obvious difficulties either of proof or of disproof , to circumvent these, it is necessary to work with changes in GFR or serum sodium which are well outside the range of error of the methods of estimation   If it can be shown that a sodium load clearly in excess of normal does not necessarily lead to a high sodium excretion, then tubular activity must be in such circumstances a determinant of sodium excretion   In these experiments we have induced a low level of sodium excretion by a low-sodium diet, and have then increased the sodium load acutely by intravenous saline

Experimental plan and methods

We aimed at producing in normal subjects a moderate degree of salt deficiency enough to diminish the urinary output of sodium to low levels, but not enough to produce gross disturbance of circulation associated with

a low plasma volume   For this purpose we relied on a diet of very low sodium content, based on the rice diet of Kempner (7), and avoided active measures of salt depletion, such as were used by McCance (11) to induce the severer grades of salt deficiency   The actual diet used was limited to rice and fruit, sweetened with refined sugar , this diet supplied not more than 0 2 gm of sodium daily, and about 1 g of potassium, and 25 g of protein   After five to six days on the diet the effect of infusing hypertonic salt solution was observed   After two to three preliminary periods, in which the base line excretion rates and inulin clearance were established, the infusion of saline was begun   The amount and rate of the infusions used in different experiments are shown in Table I   The observations on excretion rates and inulin clearance rates were continued for several periods after the infusion   Blood was collected by paraffined syringe, under oil, and the serum or plasma analysed for sodium, potassium, chloride, bicarbonate, urea, creatinine, and inulin   Urine specimens were passed into measuring cylinders, covered with paraffin, and analysed for the same substances ,   phosphate, sulphate, and pH were also determined on the urine specimens   Similar observations were made at a time when our subjects were not on the rice diet , such comparison experiments were made at the same time of day, and under the same experimental conditions, apart from the preceding diet   We have obtained fairly complete data on four normal subjects, and some data on a fifth normal subject who had vasomotor disturbances sufficient to invalidate the inulin clearance measurements   The analytical methods were as follows —

Sodium and potassium	Flame Photometer
Inulin	Colorimetric (Bacon and Bell (1))
Chloride (plasma)	Titration (Sendroy (19))
„ (urine)	Thiocyanate titration
Bicarbonate	Van Slyke manometric
Urea	Aeration and titration
Creatinine	Colorimetric (Brod and Sirota (2))
Phosphate	Molybdate colorimetric
Sulphate	Gravimetric
pH	Electrometric

RESULTS

Effects of the rice diet

All the subjects found the diet monotonous and unpalatable, and the amount consumed tended to fall off   No positive ill-effects were noted, unless perhaps a slight lethargy , when normal diet was resumed, three of the five subjects had pale bulky stools for a day or so   The data in the pre-infusion periods show that inulin clearance was lower than normal on the rice diet (Table II)   In only one subject was this difference considerable This fall in inulin clearance is in accord with previous data on hypertensive patients treated on the rice diet, Chasis (3)

As might be expected sodium excretion was much lower on the rice diet   Comparing the pre-infusion periods only, the "normal" sodium excretion in three subjects averaged 236, 249, and 327 micro-equivalents per minute (μEq /min), while the "rice-diet" excretion was respectively 44, 41, and 11 μEq /min   These data obtained on short periods agree with the 24-hr sodium excretions observed in one normal subject, which amounted on successive days from starting the rice diet to 64, 36, 47 and 12 μEq /min

TABLE I

Amount and rate of infusions

Experiment	Subject	Concentration (% NaCl)	Infusion		Total Na (mEq)
			Rate (ml /min)	Duration (mins)	
1	R P —normal	5%	8 3	24	171
2	„ —rice diet	5%	10 0	20	171
3	G M —normal	(Clearance determined without saline)			
4	, —rice diet	10%	8 0	25	342
5	D B —normal	5%	10 0	21	180
6	„ —rice diet	3 3%	1 5	78	60
7	S W S —rice diet	3 3%	1 5	78	60
8	M.D.M —rice diet	5%	7 0	50	300

TABLE II

" Normal " and " rice diet " inulin clearances

Subject	Normal clearance (ml /min.)	Rice diet clearance (ml /min.)
R P	91	85
G M	150	90
D B	101	93

Although smaller than normal, these amounts are still in excess of the sodium supplied in the diet   Within the relatively short period covered by our experimental diet, nitrogen balance remained negative , and this has been observed over longer periods by Currens *et al* (4), in spite of the claim by Kempner (7) that nitrogen balance is soon attained   The blood urea was low on the rice diet   In one subject studied by balance experiments, the urine volume alone exceeded the amount of fluid drunk, and there was a

loss of potassium from the body   Together with the negative sodium balance, these findings suggest a small loss of both intracellular and extracellular fluid , but there was no clinical evidence of dehydration

Effects of the infusion

The slow infusion of hypertonic saline (experiments 6 and 7) caused no definite change in either inulin clearance or serum sodium level   Likewise, there were no general changes during or after the infusion   The rapid infusion of hypertonic saline (experiments 1, 2, 4, 5, and 8) was attended by shivering and malaise in two of the four experiments (2 and 4), and in the actual period of infusion the inulin clearance was reduced in these experiments , in the others there was no malaise and no depression of inulin clearance   All subjects noted a salty taste or complained of thirst   The one subject to whom 10% saline was given rapidly had a thrombosis of the vein used, a complication which is not recorded by others who have given even larger infusions of 10% saline at the same rate   At the end of the rapid infusion the three subjects on the rice diet showed a rise in inulin clearance , this was absent on normal diets, but the data are not strictly comparable in respect of the amounts infused   The serum sodium increased after infusion, but the increase was small, and it is clear that the main internal change after infusion is a large increase in extracellular fluid   This expansion of extracellular fluid is the immediate defence against increase of serum sodium, and the data show how effective it is   The kidneys play little part in the early stabilisation of serum sodium, for the amounts excreted during and soon after the infusion were quite trivial in comparison with the amounts given   Further evidence of the expansion of extracellular fluid was given by the changes in plasma inulin level under conditions of constant inulin supply   These showed decreases of 20-30%, which implies an increase in " inulin space," though this cannot be applied quantitatively, as short-term equilibrium was probably not attained   The serum chloride concentrations increased more than did the sodium concentrations, and there was a fall in plasma bicarbonate   The data on inulin clearance and serum sodium are summarised in Table III , they are in general accord with the data of Mokotoff *et al* (13), and of Kriss and Futcher (9) on the infusion of 10% saline into normal people

Sodium excretion

Our main object in these experiments was to examine the thesis that sodium excretion is essentially dependent on sodium load   Sodium excretion was measured directly   We have calculated sodium load simply by multiplying inulin clearance by serum sodium, without making any correction for the Donnan effect   The systematic error involved in this method is negligible in relation to the magnitude of the changes which we induced in sodium load   We have already mentioned the low level of sodium excretion on the rice diet and this by itself might be partially

explained in terms of a slight decrease in sodium load, but increased reabsorption of sodium in the tubules stands out clearly when the sodium load is raised above normal by rapid saline infusion   In Table IV, we give the relationship between sodium load and sodium excretion in eight experiments, taking as our reference period the urine collection period immediately after the completion of the infusion   In experiments 3, 6, and 7, no large infusion was given, and here we have given the middle period of

TABLE III

Effect of large hypertonic infusion on GFR and serum sodium

Experiment	Subject	Inulin clearance (GFR) (ml /min)			Increase in serum sodium (mEq /l)
		Before	During	After	
5	D B normal	101	97	106	2
1	R P normal	91	93	85	5
2	R.P rice diet	85	57	158	4
3	G M rice diet	90	34	210	7
8	M.M. rice diet	81	73	171	9

TABLE IV

Experiment	Subject	State	G F.R ml /min	Serum Na mEq /l	Load mEq /min	Excretion μEq /min	% Reabsorption
1	R.P	Normal	83	148	12 3	434	96·47
2	R P	Rice	158	144	22 7	73	99 68
3	G M	Normal	148	140	20 7	236	98 86
4	G M	Rice	210	140	29 4	118	99 60
5	D B	Normal	106	141	14 9	241	98 38
6	D.B	Rice	103	140	14 4	62 5	99 94
7	S S	Rice	172	135	23 2	18 7	99 92
8	M.M	Rice	171	145	24 8	413	98 33

observation   In Fig 1, we have plotted the percentage reabsorption of sodium against sodium load, taking all periods in the eight experiments into account   The complete figures are given in an appendix   It will be seen that on the rice diet the percentage reabsorption of sodium ($R_{Na}\%$) is greater than on a normal diet, and that this is so quite independently of

sodium load   The observed changes in percentage reabsorption on normal and rice diets are also independent of changes in the minute volume of urine The range of urine volume covered by our results is considerable (from 0 3 ml /min to 10 7 ml /min) , water by mouth was given freely during the test, but the hypertonic saline injections were followed by notable and protracted oliguria   The high $R_{Na}\%$ in the rice diet subjects is not dependent on a low plasma level of sodium, for the saline infusions raised this to normal levels without bringing sodium reabsorption down into the normal range   The only exceptional result in our series is in the two final periods in subject M D M , experiment 8, in which massive saline infusion lowered the $R_{Na}\%$ to 98 33 and 98 58%, values which fall in the upper
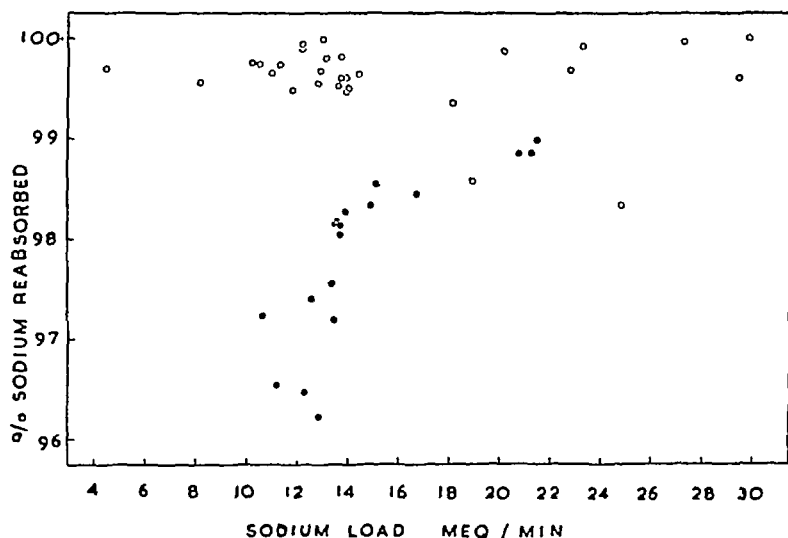

Fig 1   Relation between sodium load (mEq /min.), and sodium percentage reabsorption (as % of load)   The closed circles represent periods on normal diet, the open circles periods on the rice diet

part of our normal range   In this experiment, the infusion of 5% saline caused venous pain, and had to be slowed to a rate of less than 10 ml /min , so that the infusion lasted for an hour instead of the usual twenty minutes Thus the change from "salt-deficient" to "normal" handling of sodium load may already have begun in this subject, but his results are included in the "salt-deficient" group   The mean sodium reabsorption in 29 "rice-diet" periods was 99 61 $\pm$ 0 37% and in 18 "normal" periods 97 86 $\pm$ 0 85%   The standard error of the difference is 0 24%, and P<0 001

Although percentage sodium reabsorption is probably the most suitable measure of the way in which the kidney treats the sodium ion, from the point of view of sodium balance the important quantity is the absolute amount of sodium which is excreted   Our results show the lack of dependence of excretion on sodium load in at least two ways   Results from experiments
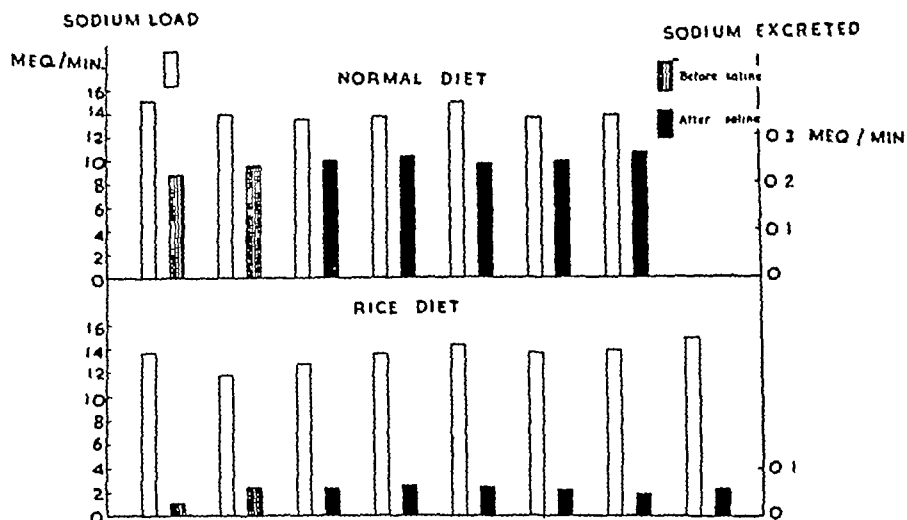
Fig 2   Data from experiment 5 (normal diet) and experiment 6 (rice diet), showing the relation between sodium load and sodium excretion in successive clearance periods   (Note —The scale of excretion is 40 times that of the load)
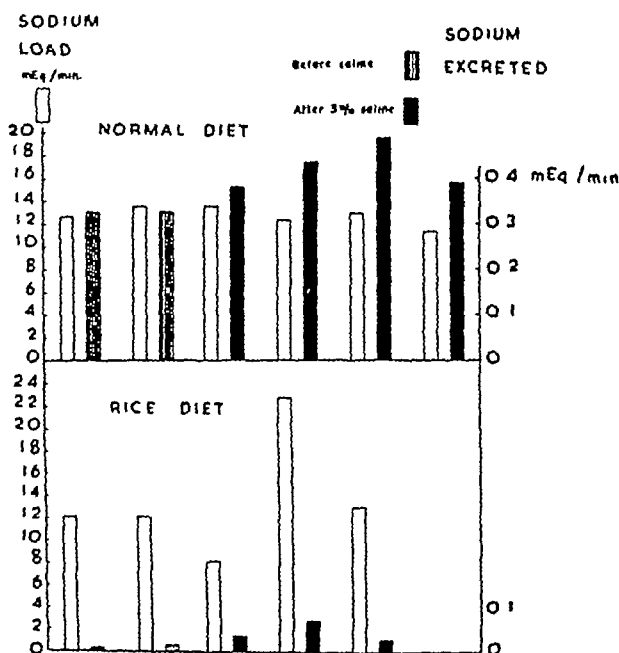

Fig 3   Data from experiment 1 (normal diet) and experiment 2 (rice diet), showing that in the latter a large increase of sodium load does not raise the sodium excretion to normal levels   Scale as in Fig 2

5 and 6 (Fig 2) show that at comparable levels of sodium load the same subject has a much lower sodium excretion after a few days on a low-sodium diet   Fig 3 shows that even when the load is increased by saline infusion, the excretion of sodium in the rice-diet experiment still does not approach the levels found in the same subject when taking a normal diet   There is, of course, some increase in sodium excretion with saline infusion in the rice-diet subjects , the narrow range of $R_{Na}\%$ necessitates this   but it is trivial in comparison with the difference between the sodium excretion in normal and rice-diet experiments
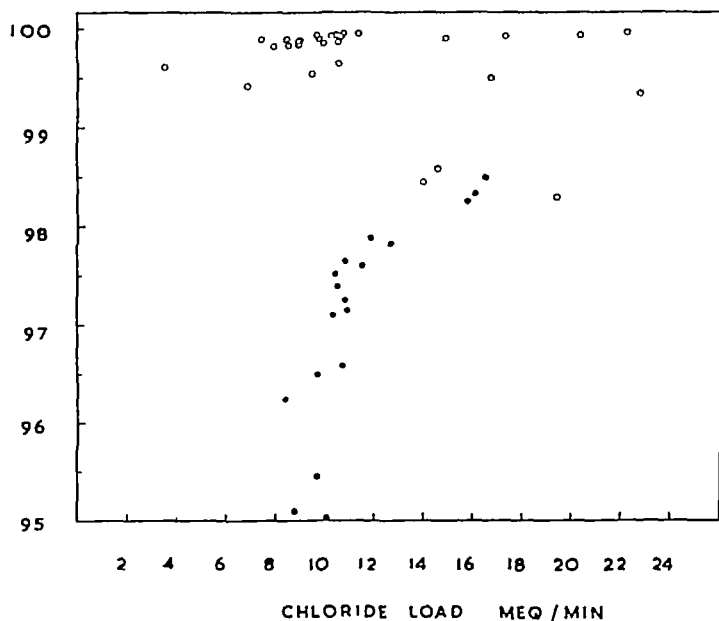

Fig 4   Relation between chloride load (mEq /min) and chloride percentage reabsorption (as % of load)   Symbols as in Fig 1

Excretion of other substances

The excretion of *chloride* was rather similar to that of sodium, both on the normal and on the rice diet   A comparison of Fig 1 and Fig 4 shows the analogy, but minor differences are also apparent   The average percentage chloride reabsorption ($R_{Cl}\%$) in the rice diet is even higher than that of sodium, but two of the three subjects exposed to a high sodium chloride load while on the rice diet showed a fall in $R_{Cl}\%$, whereas only one showed a similar fall in $R_{Na}\%$ On the other hand, the normal range of $R_{Cl}\%$ extends as low as 95%, while we have not met a normal value for $R_{Na}\%$ lower than 96 2%   The contrast between the "normal" and "rice-diet" handling of chloride is even greater than that for sodium

The excretion of *bicarbonate* was related to urinary pH rather than to the concomitant output of sodium or chloride   This must be so, since urinary pH is a function of log $\dfrac{H\ HCO_3}{B\ HCO_3}$, and $H\ HCO_3$ for tubular urine approximates $H\ HCO_3$ for tissue fluid, and so is relatively constant, so that the wide variations in urinary pH must be reflected in similar wide variations in $B\ HCO_3$ This familiar relationship was unaffected by the special conditions of our experiments

The level of *potassium* excretion was lower on the rice than on the normal diet (Table V and Fig 5)   This was so at comparable levels of potassium load, indicating that potassium reabsorption was greater on the rice diet, in spite of the fact that the potassium content of the rice-diet is within the normal range   This may be a reflection of an expanded intra-

TABLE V

Comparison of excretion rates of potassium, sulphate, and phosphate on normal and rice diet
(Data from pre infusion periods)

Experiment	Diet	Potassium excretion μEq /min	Inorganic sulphate μEq /min	Inorganic phosphate mg /min
1	Normal	112	58	0 51
2	Rice	33	23	0 29
3	Normal	140	—	—
4	Rice	93	19	0 19
5	Normal	136	25	0 81
6	Rice	30	10	—
7	Rice	55	17	0 15
8	Rice	52	—	0 20

cellular fluid compartment, but the mechanism whereby this adaptation occurs is not known to us   Another possibility, equally unproven, is that enhanced tubular reabsorption of sodium and chloride may favour potassium reabsorption   this cannot be a universal happening, in view of the opposing action of desoxycorticosterone (DCA) on sodium and potassium excretion

Our data on *sulphate* and *phosphate* excretion are incomplete (Table V) However, as one might expect from the low excretion of both sodium and potassium, the excretion of both these anions tended to be low on the rice diet, irrespective of the level of glomerular filtration   The lack of relation of sulphate excretion to inulin clearance casts some doubt on the use of sulphate clearance to measure filtration rate (Raaschou (16)) , they were certainly not equal in the conditions of our experiments

Our main object in studying the excretion of these other electrolytes in some detail was to ensure that any changes in their excretion which might affect sodium excretion should be apparent   It is clear from Table V that the low excretion of sodium and chloride in the rice diet cannot be merely a secondary consequence of a high excretion of potassium, sulphate
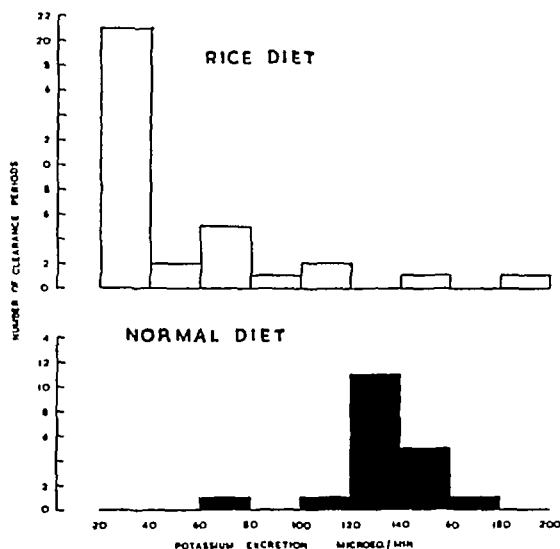

Fig 5   Comparison of rate of potassium excretion (μEq /min) on normal and rice diet frequency distribution.

and phosphate   Similarly in Table VI we have collated the changes in electrolyte excretion induced by infusion   With small infusions, there was little change in the excretion of other electrolytes, even though in experiment 7 sodium excretion rose considerably   The increase in electrolyte excretion induced by infusion was greater on the rice diet than on normal diet, with comparable infusions   With few exceptions, when a rise in sodium excretion occurred, other electrolytes were also excreted at an increased rate   The general conclusion we would draw from these findings is that in our experiments the renal handling of sodium and chloride was not seriously distorted by primary changes in the excretion of other electrolytes

The rate of excretion of *urea* increased after the infusion in experiments 6 and 7, and decreased in experiments 1, 2, 4, 5 and 8   These changes were related mainly to changes in the urine volume, which increased in experiments 6 and 7, and decreased in the other experiments   The rate of ammonia nitrogen excretion on the rice diet was low, and was not significantly modified by the infusion   The excretion of creatinine bore a general relationship to that of inulin, but in a majority of experimental periods the two clearances were not identical (Fig 6)   The urine pH on the rice diet ranged from 5 8 to 7 6, and usually fell after the infusion of hypertonic saline

TABLE VI

Ratio of post-infusion to pre infusion rates of electrolyte excretion

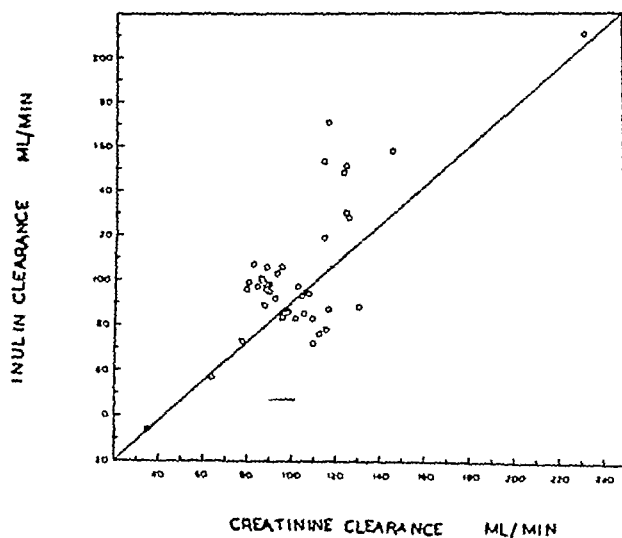| Experiment	Diet	Total infused (mEq Na)	Sodium ratio	Potassium ratio	Cl ratio	Phosphate ratio	Sulphate ratio
1	Normal	171	1 3	0 85	1 37	1 6	0 64
2	Rice	171	4 9	1 77	5 7	2 1	1 66
4	Rice	342	3 7	4 3	12 6	4 9	14 7
5	Normal	180	1 05	1 01	1 14	1 38	0 9
6	Rice	60	1 44	1 2	0 5	—	1 2
7	Rice	60	13 0	1 2	1 82	1 7	1 77
8	Rice	300	26 6	1 9	18 1	1 8	—

Fig 6   Relation between inulin clearance (ml /min) and creatinine clearance (ml /min)

DISCUSSION

Our results show rather clearly that the amount of sodium reabsorbed by the renal tubules is not determined solely by the sodium load , they are thus in contradiction to the claims of Mokotoff *et al* (13), who described a

straight-line relationship between these two quantities   Other workers have demonstrated a similar variability of tubular reabsorption under different circumstances   Thus, Newman (14) and his co-workers have found that mild exercise depresses sodium excretion in normal people, even without any perceptible change in GFR   Two groups of experiments have been reported more closely resembling our own, in that hypertonic saline infusions were used to increase the sodium load   Kriss and Futcher (9) found a fall in $R_{01}\%$ both in normals and in patients with Cushing's disease, after massive saline infusion   In dogs, Green and Farah (6) found that large changes in sodium excretion could be induced by saline infusions without any consistent or proportionate changes in GFR   As a demonstration of the independent variability of tubular function, the results reported here have certain advantages over the reports cited from the literature

(1) Thanks to the larger hæmodynamic changes produced by rapid saline infusion in our rice-diet subjects, we were able to study sodium excretion over an unusually wide range both of GFR and of urine volume

(2) For the same reason, the changes in GFR were unequivocal in the loading experiments done on the rice diet

(3) Our experiments were all carried out at the same time of day, the forenoon, in which sodium excretion is maximal in the normal person (Norn, (15), Stanbury (20))   This makes the differences in sodium excretion between the " normal " and " rice-diet " subjects more definite, but at the same time strictly comparable in this respect

(4) There are enough results on the excretion of other substances to allow us to exclude any serious bias of sodium excretion by gross changes in the excretion of other substances   In particular, we can exclude osmotic diuresis as an explanation of the changes in sodium excretion which we observed after infusion , for the excretion of other substances tends to rise along with that of sodium in our results, whereas in osmotic diuresis the excretion of substances other than that causing the diuresis falls, and the urine becomes more dilute (McCance (12), Rapoport *et al* (17))

In considering what may be the explanation of our results we must distinguish between the possible stimulus to sodium excretion and the mechanism by which this stimulus becomes effective   While the amount of salt infused may not have replaced completely the amount lost during the first few days of the rice diet, it was at least sufficient to raise the plasma sodium and it must have considerably expanded the plasma volume and extracellular space   None of these stimuli was sufficient to abolish the high rate of tubular absorption of sodium which had occurred on the low-sodium diet but we cannot on this evidence exclude any of these factors as the normal stimulus to sodium excretion because the stimulus may act through a mechanism which may take time to come into operation, *e g* , a hormonal mechanism

If the mechanism which adjusts sodium excretion to body needs does in fact involve a time-lag, then the arguments which we have already given against GFR being the direct controlling factor of sodium excretion are reinforced, since there can be no time-lag in variations of sodium load produced by this means   This applies not only to conventional variations in GFR, with a constant number of glomeruli perfused, but also to variations attended by opening or closing of glomeruli   On the rice diet, it is conceivable that the low GFR might be associated with a smaller number of active nephrons , but at any given level of GFR this would be expected to diminish $R_{Na}\%$, and could not account for the high sodium reabsorption which was in fact observed

Our results are hard to reconcile with two concepts put forward by Wesson *et al* (22)   As already stated, they place emphasis on variations in sodium load as the main determinant of sodium excretion , we have shown that our results cannot be explained in this way   Less directly, their other concept of a maximum of distal tubule reabsorption of sodium is deprived of physiological significance by our demonstration that after a few days on the rice diet the tubule will perform feats of reabsorption which are never likely to be demanded of it in everyday life

As with the initiating cause of sodium retention, so with its mechanism we have no direct lead from our results, unless it be that the slowness with which the effect passes off favours a hormone mechanism   Changes in pituitary and in adrenal cortical activity have to be considered   Surprisingly, data on the effect of pituitrin on sodium, as opposed to water, excretion are scanty and inconsistent   On the whole, pituitrin is thought to increase sodium excretion but there has been no convincing demonstration that this is the case in normal man   Until further information is available on this point, it is futile to speculate on the possible role of ADH in producing the observed results   It can be said, however, that pituitary action was not maximal on the rice diet, for hypertonic saline injection was followed by a fall in urine volume   It is equally difficult to define the part which might be played by the adrenal cortex in producing the phenomena we have observed   Steroids of the desoxycorticosteroid group are certainly salt-retaining, and œstrogens and androgens also lead to salt retention , on the other hand, corticosterone and related compounds may produce at least a temporary increase in sodium and chloride excretion (Kendall (8))   Leaf and Couter (10) have contended that the renal excretion of sodium in normal subjects is regulated by a DCA-like substance   We find two possible difficulties in attributing our observed pattern of renal electrolyte excretion to a DCA-like substance —

(1) There was no change in serum potassium, and *no* enhanced potassium excretion, in subjects on the rice diet

(2) We found great difficulty in inducing water diuresis when our subjects were on the rice diet Although DCA is salt-retaining, it enhances water excretion, in antagonism to pitressin (Sartorius and Roberts (18)) , these results were obtained in dogs, however, and may not apply in man

It is possible, of course, that the action of DCA in enhancing water and potassium excretion may be corrected by other steroids when the adrenal acts as a physiological unit We have recently observed very marked retention of sodium, in response to ACTH, without any definite change in potassium excretion Moreoever, Gaudino and Levitt (5) have found that DCA and whole adrenal cortical extract differ in their effect on water-balance , DCA produces no change or a decrease in body-water, whereas whole cortical extract causes considerable expansion of intracellular fluid and body-water We are inclined to attribute the sodium retention observed in our salt-depleted subjects to an over-action of the adrenal cortex as a whole, the action of DCA-like substances being substantially modified by that of other steroids

SUMMARY

1 Five normal subjects were given a rice-and-fruit diet containing less than 0 2 g of sodium per day After five days, the excretion of sodium in the urine was less than $50\,\mu Eq$ /min , the excretion in the same subjects on a normal diet at the same time of day was over $200\,\mu Eq$ /min

2 When hypertonic saline (5 or 10%) was infused at rates up to 10 ml /min , the sodium excretion remained less than normal, even although such massive infusions were followed by a large increase in sodium load (plasma sodium $\times$ GFR)

3 The very low excretion of sodium on a salt-poor diet was mainly attributable to an increased tubular reabsorption of sodium, only a minor part being played by the diminished sodium load The mean sodium reabsorption in 29 "rice-diet" periods was 99 61 $\pm$ 0 37%, while on a normal diet (18 periods) it was 97 86 $\pm$ 0 85%

4 The increased sodium reabsorption found in the rice diet subjects persisted after the plasma sodium and the inulin clearance had been raised to normal levels, or higher, by saline infusion This shows that sodium excretion cannot be regulated solely by changes in GFR and plasma sodium

5 The delay in the readjustment to a normal or increased sodium load suggests that a hormonal mechanism may be concerned , and there might be an over-production of adrenal cortical hormones during salt depletion Our results do not define the stimulus in response to which such an adjustment is made

APPENDIX

Experimental data on urine volume, GFR, and sodium excretion

Experiment 1   Subject R P   M   49 years   Normal diet   Infusion of 5% NaCl in period 3
Total sodium given—171 mEq

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (μEq /min)	Sodium reabsorption %
1	26	3 0	88	143	12 6	327	97 4
2	33	8 6	94	143	13 4	326	97 57
3	45	5 6	93	—	13 5	380	97 19
4	33	2 0	83	148	12 3	434	96 47
5	32	2 0	87	—	12 9	485	96 23
6	36	1 7	76	148	11 2	389	96 54
7	45	1 3	72	—	10 7	295	97 23

Experiment 2   Subject R P   Rice diet for six days   Same infusion as in experiment 1, given in period 3

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (μEq /min)	Sodium reabsorption %
1	68	1 7	85	143	12 2	7	99 94
2	23	5 7	85	—	12 2	15	99 88
3	36	3 5	57	144	8 2	36	99 56
4	20	4 3	158	147	22 7	73	99 68
5	93	0 5	89	147	13 1	26	99 80

Experiment 3   Subject G.M   M , 34 years   Normal diet   No infusion

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min.)	Sodium excretion (μEq /min.)	Sodium reabsorption %
1	16	4 4	119	—	16 7	258	98 45
2	29	2 0	151	140	21 2	240	98 86
3	21	5 9	148	—	20 7	236	98 86
4	20	6 1	153	—	21 4	218	98 98

Experiment 4   Subject G M   Rice diet for five days   Infusion of 10% NaCl in period 3
Total sodium given—342 mEq

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (μEq /min)	Sodium reabsorption %
1	25	10 7	83	133	11 0	39	99 65
2	33	3 1	97	—	12 9	42	99 67
3	66	0 3	34	133	4 5	14	99 69
4	43	2 1	210	140	29 4	118	99 60
5	29	1 1	128	141	18 1	115	99 36

Experiment 5   Subject D B , M , 36 years   Normal diet   Infusion of 5% NaCl in period 4
Total sodium given—180 mEq

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (μEq /min)	Sodium reabsorption %
1	18	1 1	107	141	15 1	219	98 54
2	32	1 2	99	140	13 9	241	98 27
3	24	1 3	96	—	13 5	249	98 15
4	24	1 1	97	—	13 7	257	98 13
5	20	1 1	106	—	14 9	241	98 38
6	21	1 1	95	142	13 5	246	98 16
7	48	1 1	96	—	13 7	264	98 04

Experiment 6   Subject D B   Rice diet for five days   Infusion of 3 3% NaCl in periods 3, 4,
5, 6   Total sodium given—60 mEq

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (μEq /min)	Sodium reabsorption %
1	27	0 4	101	136	13 7	26	99 8
2	26	0 6	86	—	11 8	61	99 48
3	22	3 0	93	138	12 8	59	99 54
4	26	4 6	98	138	13 6	64	99 52
5	19	5 7	103	140	14 4	62	99 64
6	18	5 6	99	138	13 7	56	99 59
7	13	5 2	100	—	13 9	47	99 46
8	11	5 2	106	141	14 9	57	99 60

Experiment 7   Subject S W S , M , 30 years   Rice diet for five days   Infusion of 3 3% NaCl
in periods 2, 3 4   Total sodium given—60 mEq

Period	Duration of period (min)	Urine volume (ml /min)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (µEq /min)	Sodium reabsorption %
1	60	0 4	96	136	13 0	1	99 99
2	43	1 1	221	133	29 8	2	99 99
3	24	6 3	202	—	27 2	13	99 95
4	16	8 1	172	135	23 2	19	99 92
5	21	9 2	148	136	20 1	26	99 87

(*Note.*—Pallor and faintness occurred in period 1, after a drip had been set up, but before saline
infusion)

Experiment 8   Subject M.M , M , 34 years   Rice diet for five days   Infusion of 5% NaCl in
periods 3, 4, 5   Total sodium given—300 mEq

Period	Duration of period (min)	Urine volume (ml /min.)	Inulin clearance (ml /min)	Plasma sodium (mEq /l)	Sodium load (mEq /min)	Sodium excretion (µEq /min)	Sodium reabsorption %
1	16	7 3	78	135	10 5	27	99 74
2	22	10 0	83	—	11 3	31	99 72
3	31	9 0	73	136	10 2	25	99 75
4	23	9 0	98	—	14 0	69	99 5
5	45	4 1	171	144	24 8	413	98 33
6	29	2 7	130	145	18 0	268	98 58

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HYPERTENSION PRODUCED IN THE RABBIT BY LONG CONTINUED INFUSIONS OF RENIN

By R B BLACKET,* A DEPOORTER,† G W PICKERING, A L SELLERS‡ and G M WILSON

It is now very widely believed that renin is the agent responsible for hypertension in the initial period, which may last days or weeks, after renal artery constriction In the dog experimental renal hypertension is independent of the nervous connections of the kidney (2), is accompanied by an excess of renin and hypertensin in the blood (3, 8), and is abolished by removing the ischæmic kidney (6, 2, 33) In the rabbit experimental hypertension is accompanied by an increase in the renin content of the kidney (26) and is abolished by nephrectomy in a similar time to that taken for hypertension to subside after stopping an infusion of renin (14) But in the later stages it is uncertain how far renin is concerned in the maintenance of hypertension In the dog, present evidence is against an increase in the renin content of the blood (12), nephrectomy may or may not abolish hypertension (29) In the rabbit, the renal content of renin is not increased (26), nephrectomy fails to abolish hypertension (23)

In trying to judge the role, if any, of renin in the genesis of experimental hypertension, a major difficulty is the dearth of knowledge concerning the characteristics of hypertension produced by the release of renin into the blood stream It is even doubtful whether the continued discharge of renin into a vein would result in a maintained hypertension Tigerstedt and Bergman (32) showed that, at least in the anæsthetised animal, rapidly repeated injections lead to progressive decrease and final extinction of the response, a phenomenon which has been fully confirmed Page (22) found that continued intravenous infusion of renin failed to produce maintained

hypertension in anæsthetised dogs   On the other hand Hill and Pickering (14) found that small doses of renin infused into the ear veins of unanæsthetised rabbits for four hours would produce a maintained hypertension of about 30 mm Hg , the arterial pressure took about 4 hours to return to normal after stopping the infusion   With larger doses, hypertension was not maintained   Taggart and Drury (31) obtained similar results   It seemed probable therefore that in the unanæsthetised rabbit hypertension could be maintained indefinitely, provided a suitable dose of renin was used and satisfactory methods devised for its prolonged infusion   This has been done and we record here some of the properties of hypertension produced by known doses of renin

Methods

At the outset it was clear that there would be many technical difficulties in obtaining a suitable preparation of renin and in devising an arrangement whereby this could be delivered at a constant rate into a vein for long periods while the animal was unanæsthetised and relatively unrestrained   These were gradually overcome   The final difficulty shows how narrowly we succeeded   Even when we had prepared what seemed to be a suitable and sterile solution of renin, and devised a method for delivering it at a steady rate by a sterilised apparatus, our animals fell ill and died   Careful analysis showed that this toxic effect was due to a bacterial reaction taking place in the infusion apparatus,   It was only by taking the strictest aseptic precautions that it was possible to maintain the animals in good physical condition and to produce persistent hypertension

Renin

Since renin is a protein and is antigenic (8, 18), it is essential that the preparation used should be made from the kidney of the same species as the animal into which it is to be injected We have been fortunate in obtaining the kidneys from some 2,000 rabbits within 18 hours of death by trapping, and in obtaining the kidneys from the frozen carcasses delivered to the Hospital kitchen   Preparations from these kidneys have been made by the following two methods

1   *Alcohol method*   The majority of preparations were made by this method, which has the advantage that the alcohol-dried kidney powder is stable and can be worked up as required

The kidneys are stripped of fat and connective tissue, and minced   Ethanol is added in the proportion of 2 ml. per g   and the mixture placed in the refrigerator for 2 days during which it is shaken or stirred repeatedly   It is then filtered through muslin, as much alcohol as possible expressed by squeezing, and the residue dried at room temperature   The dry powder is stored and used as required   About 400 g of powder is extracted with 4 litres saline in the refrigerator for 2 days and filtered through muslin.   The filtrate is acidified with N HCl to pH 4-4 5 and centrifuged.   The supernatant is half saturated with ammonium sulphate and left in tall cylinders for 2 days at 0°C   The resulting precipitate is separated by gravity and resuspended in half saturated ammonium sulphate   This is repeated twice and the precipitate finally separated by filtration   The precipitate is dissolved in water and the filter paper extracted with water, and the combined solutions dialysed against tap water   8 g NaCl per 100 cc are added and N.HCl to pH 4   The precipitate is centrifuged off and the solution again dialysed   NaCl is added to 0 9%, and Norit 1 mg per ml , the solution is centrifuged, and filtered through a Whatman No 42 paper

2   *Heating method*   Fresh kidneys are ground with sand in a mortar and extracted with 10 cc saline per g kidney   The extract is left in the refrigerator overnight and filtered through cloth   The turbid filtrate is heated in 400 cc lots to 56°C for 20 minutes in a water bath (20), cooled under the tap and, when cool, centrifuged   The supernatant is acidified to pH 4 with N HCl and centrifuged   The supernatant is adjusted to pH 5 6 with N NaOH and filtered The filtrate is half saturated with ammonium sulphate and left in tall cylinders in the refrigerator The precipitate is washed twice with half saturated ammonium sulphate, and finally filtered through paper   The precipitate is suspended in water, to which are added washings of the filter paper with N/100 Na$_2$CO$_3$ The whole lot is dialysed against tap water for 24 hours and centrifuged   Sodium chloride to 8% is added to the supernatant, and the precipitate centrifuged off The supernatant is brought to pH 4 5 with N HCl and the precipitate again centrifuged off After dialysis for 24 hours against tap-water, the solution is made up to 0 9% with solid NaCl, treated with Norit 1 5 mg per cc , filtered twice through Whatman No 42 paper in the refrigerator

Sterilisation of renin Renin cannot be sterilised by heat It is destroyed by boiling and by keeping at 56°C for 2 hours Katz and Goldblatt (19) have sterilised it by Seitz filtration, but after this procedure our preparations, in small samples, have shown complete loss of activity, probably due to adsorption on the asbestos pads At the suggestion of Mr Holt and Dr Himmelweit, filtration through a graded collodion membrane of pore size 0 81µ was tried, and produced without loss of potency a bacteriologically sterile solution With the help of Dr Himmelweit and his staff in the Wright-Fleming Institute, filtration through membranes of pore size 0 6 to 0 81µ has been used with consistent success to sterilise our preparations of renin

Assay of renin. The solutions used have been assayed against a standard powder using the response of the unanæsthetised rabbit (24) The unit used is that originally proposed (24), namely the renin content of 100 mg of the alcohol dried, rabbit kidney powder used as standard and not as is frequently misquoted the amount of renin required to raise the blood pressure of an unanæsthetised rabbit by a certain amount The original standard came to an end with war in 1939 The standard used here was prepared from a large batch of rabbits' kidneys and was assayed against some powders that had been standardised by the original standard and had been kept in the refrigerator for some eight years The unit used is thus as close to the original unit as we could insure

Protein content of the renin preparations The protein content of 4 of our renin preparations was estimated by Dr A H James using the micro Kjeldahl method with the following results,

48K (alcohol method) 0 04 mg N per unit activity

49B (alcohol method) 0 07 mg N per unit activity

49G (alcohol method) 0 04 mg N per unit activity

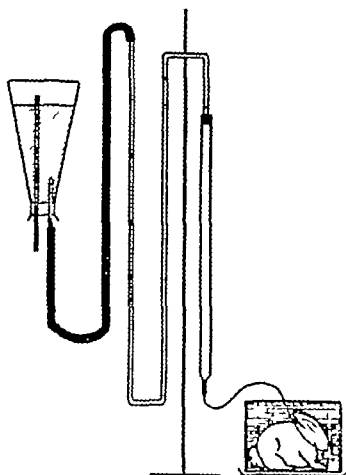49H heating method 0 03 mg N per unit activity

Fig 1 Diagram of the apparatus used for prolonged continuous infusion in the rabbit

Method for continuous intravenous infusion

The delivery apparatus We found the mechanically driven syringe that we tried unsuitable, since the motion of the plunger was discontinuous permitting reflux of blood into the venous canula with eventual clotting We therefore had recourse to the apparatus originally designed by Burn and Dale (4) which had previously been successfully used for short infusions in the rabbit The apparatus used in our experiments is shown in diagrammatic form in Fig 1 The four components can be autoclaved separately, and be easily assembled with aseptic technique An inverted litre conical flask (1) acts as a reservoir and is nearly filled with heavy oil * The flask is fitted with (2) a rubber bung carrying two glass tubes one to admit air is fitted with a wool filter, the other to transmit the oil is joined to about 4 feet of pressure tubing The pressure tubing passes to (3) a thick walled glass capillary tube 4 feet long and 2 mm bore, this tube is bent 4 times at right angles as shown in the figure, and carries a rubber bung which is fitted flush

* Castrol Gear Oil was used

with the end of the capillary   The bung fits into the top of (4) a 100 cc burette carrying a rubber tube terminating in a record fitting adapter, a screw clip is applied to the rubber tube   An Andrewes needle intervenes between the adaptor and the catheter   The whole apparatus is carried on upright steel rods $\frac{1}{4}$ inch in diameter and 5 feet long   Four uprights can be screwed into a steel base place, 1 foot square and made rigid by joining with horizontal rods at their tops Two sets of infusion apparatus can be conveniently carried by such a system

The catheter   After experimenting with glass and polythene canulæ in the ear veins we found that a gum-elastic ureteric catheter (size 10) tied into the external jugular vein was much more satisfactory

Procedure for infusion   The 4 components of the apparatus are sterilised by autoclaving The gear oil is sterilised by heating to 100° in the reservoir flask (1) and cooled.   The bung (2) is fitted to the flask, and the attached rubber joined to the capillary tubing (3)   The reservoir and glass tubing are now clamped to the steel uprights   Air under 100 mm Hg   pressure is forced into the reservoir   When oil issues from the free end of the capillary (still sterile and enclosed in a wool plugged boiling tube), the rubber tubing is clamped and the source of air pressure discon nected   The 100 cc burette is now filled with sterile saline containing 250,000 units of penicillin, and topped up with sterile oil   The boiling tube surrounding the glass capillary being now removed and the oil filled tubing unclamped, the burette can be pushed onto the bung and clamped to the upright   When saline begins to drip from the bottom, the tube attaching adapter to burette is clamped and the infusion apparatus is ready

The ureteric catheter filled with saline which is kept renewed from a 10 cc syringe is now inserted into one external jugular vein of a male rabbit under nembutal anæsthesia   The great and posterior auricular nerves to the left ear are cut at the same operation (subsequently, injections are made into and blood samples obtained from this denervated ear)   The catheter is carried subcutaneously to the back of the neck and then is buried in the fur for half the length of the back, by clipping the fur and bringing the mobile skin together over the catheter with two stitches The rabbit is put in a cage 14$\frac{1}{4}$ ins by 8$\frac{1}{4}$ ins by 8$\frac{1}{4}$ ins , the catheter brought through the lid of the cage and the Andrewes needle removed from the syringe and attached to the now freely dripping adapter on the burette   The whole procedure of assembling the apparatus, inserting the catheter and attaching it to the burette must be done with strict aseptic precautions

When the rabbit recovers from the anæsthetic, a copper sheet on four wooden legs is inserted into the cage, reducing the space in which the rabbit is free to move to little more than its bulk On the other side of the copper plate is an electric lamp which is lit from time to time to keep the animal warm enough to dilate fully the ear vessels, so that readings of arterial pressure can be obtained and the heart rate counted

The height of the reservoir is adjusted so that the rate of delivery from the burette is approximately 4 ml per hour   With such rates, the burette needs changing once every 24 hours This is done aseptically by first filling the new burette with the solution to be infused, detaching the old burette from the capillary, and attaching the new one after flaming the bung   After the first 24 hours, 1,000 units of heparin is added to each 100 cc of infusion in addition to the penicillin   If heparin is added in the first 24 hours a hæmatoma frequently forms in the wound and subsequently becomes infected   Clotting may occur in the catheter or in the vein   This may be overcome by forcing saline through the catheter with a syringe   It may be found however that the reservoir has to be lifted higher and higher until finally the rate cannot be maintained.   In this event the only way of maintaining the experiment is to anæsthetise the animal and insert a new catheter into another vein.

During the experiment the animals were fed thrice daily with oats and bran and greenstuff In the later experiments they were also provided with a water bottle   The cages were cleaned each day and the animals allowed to move about freely at that time

The arterial pressures were measured and the pulse rate counted on the central artery of the flushed ear of the warm animal using the capsule described by Grant and Rothschild (11)   The values obtained by this method agree, to within a few mm.Hg , with the values of mean arterial pressure obtained by a mercury manometer from a canula in the femoral artery, this corre spondence holds also during the rise of pressure produced by renin (24)

The response to a single intravenous injection of renin, using 0 5 cc of a solution made by extracting 1 g of our standard powder with 10 ml of saline for 24 hours, was tested during the infusion of renin and during the preceding and succeeding infusions of saline

RESULTS

1   *Saline Infusions*

Four animals received saline infusions continuously for 10 to 14 days Cultures from the burettes remained sterile in each case   These infusions contained the same amounts of heparin and penicillin as did the renin

infusions, were given in precisely the same way and at the same rate   They
thus served as controls to show the changes produced by the experimental
procedure without the administration of renin   An example is shown in
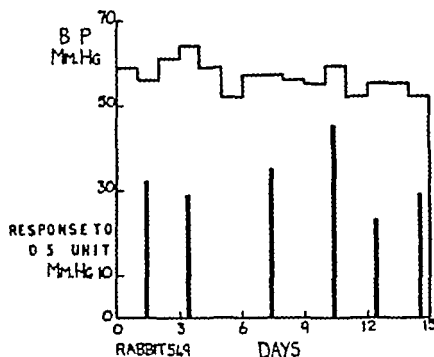Fig 2, which records the average of all the readings of arterial pressure on

Fig 2   Records the average arterial pressure for each of 15 days during which rabbit 549 received
an infusion of saline at 4 ml per hour   The black lines at the bottom show the sizes of the
responses to a single intravenous injection of 0 5 unit renin

any particular day, and the responses to intravenous injection of 0 5 unit
of renin on alternate days   In this experiment, there was a slight tendency
for the arterial pressure to fall   In the remaining animals no such tendency
could be made out , the daily average of arterial pressure varied irregularly
over a range of up to 12 mm , but showed no systematic trend   In no animals
was there any evidence of a rising pressure   In the experiment illustrated,
the response to a single injection of renin, though variable, showed no
systematic change   All these animals remained well, eating avidly and
moving actively about their cage whenever opportunity offered   In spite
of this, some of our animals lost weight

2   Renin infusions

Each animal was given saline for one or two days before the renin
infusion, and in most animals this was repeated for one or two days after
renin was stopped   It has been mentioned already that in our earlier
experiments with renin infusions, the animals after one or two days became
listless and lost their appetite, and after a day or two died   In these animals
hypertension, present initially, declined with the first signs of illness and was
often totally abolished before the animals died   This illness, and its effect
on the response to renin, was traced to growth of microbes (chiefly E Coli
and relatives) in the burettes , renin being a protein provides an excellent
culture medium   When a satisfactory aseptic technique had been put into
practice, we had no further trouble, except with one preparation made by

TABLE I

Summarises the renin infusions that were kept aseptic throughout. Renin preparations 49F and 49H were made by the heating method, the remainder by the alcohol method

Rabbit	Renin	Basal B P Beginning	Basal B P End	Range of dose Least	Range of dose Greatest	Max B P Dose units/hr	Max B P B P	Days B.P raised above base line by mm.Hg 0–9	10–19	20–29	30–39	40–49	Duration renin infusion and hypertension Days	Blood urea Beginning mg/100 ml	Blood urea End mg/100 ml
554	48H	69	63	0.8	1.8	1.8	88	0	7	0	0	0	7	—	—
501	48H	57	57	0.0	5.0	5.0	101	0	0	2	12	3	17	36	55
562	48H and K	68	64	0.85	9.25	8.25	106	0	7	7	4	0	18	34	42
550	48L	57	55	0.5	16.5	8.2	101	0	5	7	3	0	13	58	45
590	49B	64	65	1.3	7.8	7.8	128	0	0	0	10	1	11	—	—
605	49E and C	60	58	1.8	4.2	2.0	101	0	1	5	1	0	7	—	44
606	49E	63	45	2.0	6.0	6.0	80	0	4	2	0	0	6	—	44
609	49F	60	—	0.8	1.5	1.5	87	1	4	0	0	0	5	—	—
	49B	—	—	2.3	4.5	4.5	98	—	1	1	—	—	2	—	—
615	49H	63	52	1.7	5.0	5.0	107	0	2	4	3	0	9	—	—
569 (1)	48K	61	57	0.7	12.0	10.2	109	0	3	2	4	2	11	41	61
1 kidney removed															
569 (2)	48M	56	50	0.5	8.0	5.1	104	0	3	5	0	1	15	80	63
1½ kidney removed															
566	48M	73	60	1.0	4.1	3.25	115	0	3	1	3	0	7	70	155
567	48M	78	—	1.2	14.0	11.2	120	4	6	3	5	0	14	47	186
583	49O	95	73	0.83	6.0	2.25	115	4	0	0	0	0	13	43	50
585	49E	78	57	1.1	2.5	2.5	104	1	4	1	0	0	6	32	40

Before the reduction of renal substance, the arterial pressure averaged 70 mm Hg in 566, 65 in 583 and 68 in 585. The infusions were begun 8 days in 567, 22 days in 566, 28 days in 583, and 31 days in 585 after amputating the pole of the second kidney

the alcohol method which became heavily infected at an advanced stage of purification and which contained a toxin not separated from renin by ammonium sulphate precipitation and dialysis

With the remaining 9 preparations of renin, the animals remained lively, ate avidly, and in general seemed in good health   Most of our animals lost weight, but some of this may have been due to inadequate feeding or dehydration, as in the earlier experiments we relied on the fluid given by burette and contained in the food , in the later experiments water was given freely and the loss of weight was less   Although we did not systematically collect clean urine samples, those specimens we did obtain during the infusions contained protein

Fifteen infusions were carried out on 14 rabbits with 9 preparations of renin, 7 obtained by the alcohol, 2 by the heating method   The results are summarised in Table I   The previous experience of Hill and Pickering had shown the importance of dosage in determining whether or not hypertension is maintained with constant infusions of renin   It was therefore our practice to begin with doses of the order of 0 5 to 1 0 unit per hour, then to increase until a satisfactory hypertension was obtained, or to study the effects of varying dosage   In each animal the pressure rose within a few minutes of the estimated entry of renin into the vein and, as Hill and

TABLE II

Shows the changes of arterial pressure during an infusion of renin over several days at rates showing relatively small variations

Animal	Infusion at relatively constant rate			Total duration infusion days	Final base line compared with initial
	duration days	Range of dose units/hour	Trend of B P		
554	6	1 4 to 1 8	Slight fall	7	Fall
561	12½	1 5 to 2 3	Steady	17	No change
566	4	0 9 to 1 1	Slight rise	7	Fall
569 (2)*	8	1 5 to 3 0	Variable with dose	15	No change
559	4	0 6 to 1 0	No change	13	No change
569 (1)*	4	0 9 to 1 9	No change	11	No change
567	6½	1 5 to 3 0	No change Varies with dose	14	—
615	4†	1 7 to 2 7	No change	9	Fall
600	4†	0 8 to 1 5	No change	7	No change
562	11	1 1 to 1 6	Steady	18	Slight fall

  * 569 (1)   Intact animal.
        (2)   after left nephrectomy
  † Renin prepared by heating method

Pickering (14) found, often rose temporarily to a height a little above that ultimately sustained   In each case the pressure remained raised till the infusion of renin was terminated 6 to 18 days later   With saline for 2 days before and 2 or 3 days after the renin, this made our longest continuous
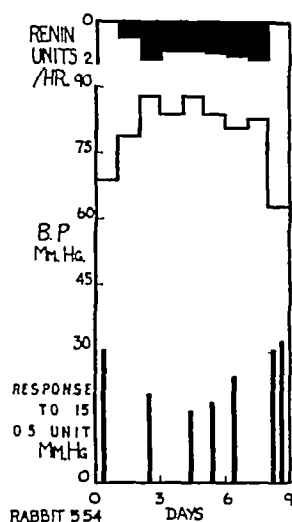

Fig 3   Rabbit 554   Shows the daily average arterial pressure produced by an infusion of renin at a relatively constant rate for 7 days   The responses to a single intravenous injection of 0 5 unit renin are shown in the bottom of the figure
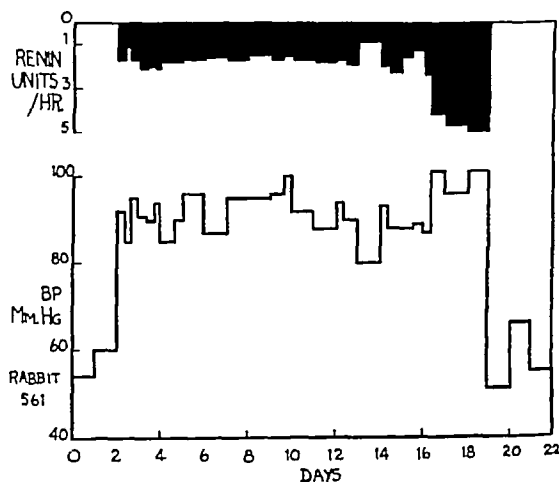
Fig 4   Rabbit 561, shows the daily average arterial pressure during an infusion of renin lasting 17 days   During the first part of the infusion the dose was kept relatively constant, during the later part it was increased   Details of the fall of pressure after stopping renin in this rabbit are given in Fig 11

infusion 24 days   Only three factors prevented the infusion being maintained longer, thrombosis of veins, supply of renin and the time that each experiment demands of the worker

The effect of a constant dose of renin In 10 experiments on 9 animals, the dose was kept within relatively narrow limits for 4 days or longer Table II summarises and Figs 3 and 4 and 5 illustrate the results obtained in these animals It has already been mentioned that in animals receiving infusions of saline the arterial pressure fluctuates, the mean values obtained for each day varying by as much as 12 mm Hg , though in some animals the fluctuations are much less Inspection of the figures obtained in the 10 experiments
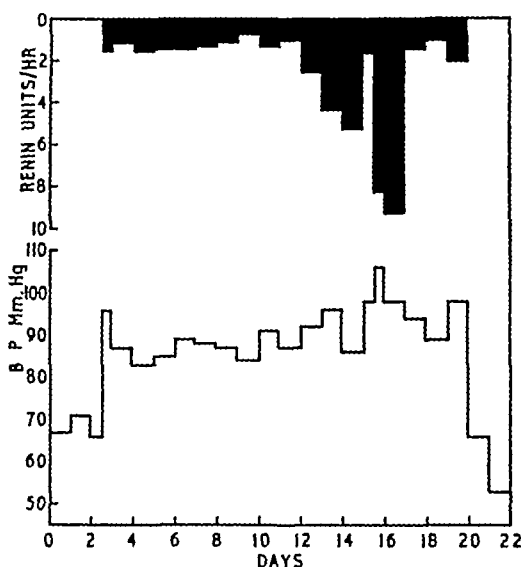
Fig 5   Rabbit 562   Shows the daily average pressure during an infusion of renin lasting 18 days

in which renin dosage was maintained relatively constant shows a definite tendency towards a fall in one animal, towards a rise in another, while in 7 animals the levels fluctuated within the 12 mm limit established in the controls but showing no definite trend up or down , in the two animals whose dosage showed the greatest fluctuation the level of blood pressure varied with dose outside these limits In the two animals receiving relatively constant dosage for the longest periods there was more definite evidence that the level of hypertension did not change with time In rabbit 561 in which dosage ranged only from 1 5 to 2 3 units per hour for 10 consecutive days, and was in the same range on the 12th and part of the 13th and 14th days, the regression line of arterial blood pressure relative to time was calculated and was found to have a negligible slope relative to the time axis or base line In rabbit 562, after 3 days saline, the dose of renin was kept between 0 85 and 1 6 units per hour from the 4th to the 13th day, then raised as high as 9 25 units per hour and again lowered to 1 5 units on the 20th day, saline being substituted on the 22nd day and the animal

killed on the 24th day   The constancy of the pressure for a given dose is well illustrated in this animal   The dose was 1 1 to 1 2 units per hour on 4 days, the 5th, the 10th, the 13th and the 21st , on those days the average arterial pressures were 86, 87, 85, and 89 mm Hg

We conclude therefore that when renin enters the veins of the rabbit at a constant rate of the order of 1 to 3 units per hour, the hypertension produced is also constant and shows no fixed trend up or down, at least over periods of the order of 17 days, and except for the rather high figures in the first few hours of infusion
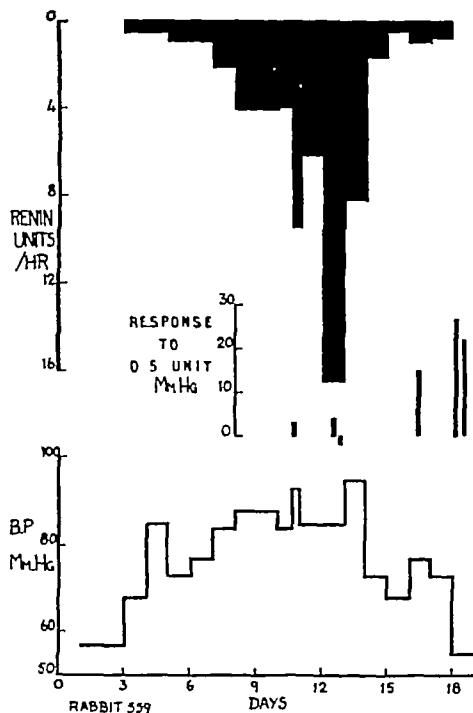

Fig 6   Rabbit 559   Shows the daily average pressure during an infusion of renin lasting 15 days   In this rabbit the dose of renin was increased stepwise and decreased stepwise   The dose response curve is shown in Figs 6 and 8   The responses to a single intravenous injection of 0 5 units renin are also shown.

The effect of varying the dose   In 11 experiments the dose was varied fourfold or more, not by varying the rate of infusion but by varying the concentration of renin in the solution infused   The minimum rate of renin dosage used was 0 5 and the maximum 16 5 unit per hour   In some of the experiments the rate of renin infusion was increased to high levels during the latter part of the experiment (Figs 4 and 5)   In others the rate was kept approximately constant for one to two days at a time and increased stepwise towards a maximum, and then decreased in a similar manner An example is shown in Fig 6, which illustrates the extreme limits of dosage

investigated   These figures all show that equal increments of dosage are associated with progressively smaller increment of arterial pressure   There seems to be a limit above which it has proved impossible to push the pressure by increasing the dose of renin   In fact in the experiment illustrated in Fig 6, the arterial pressure with the largest dose was below the maximum reached, and at this point as will be seen from the figure the response to a single intravenous injection of renin was suppressed
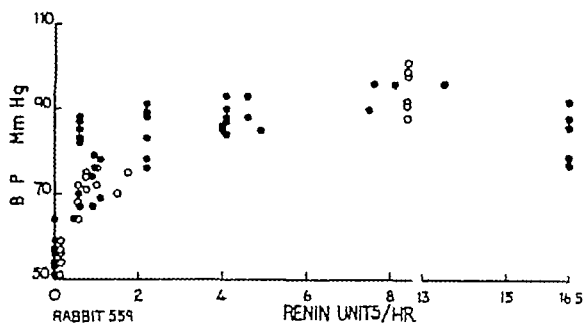

Fig 7   Rabbit 559   Shows the relationship between the height of the blood pressure and the rate of dosage of renin infused   Solid circles values on the curve of ascending dosage, open circles values on the curve of descending dosage
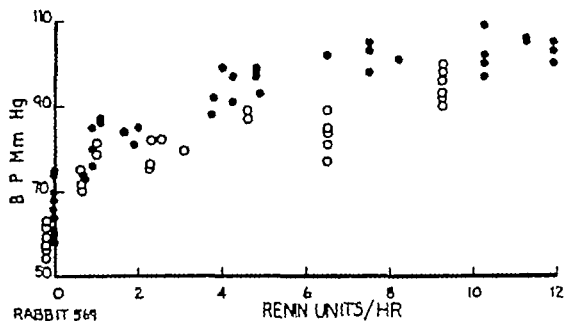

Fig 8   Rabbit 569   Dose response curve as in Fig 6   Note the difference between the values on curve of ascending dose (solid circles) and descending dose (open circles)

The relationship between dose and response may be considered in more detail in rabbits 559 (Figs 6, 7 and 9) and 569 (Figs 8 and 10)   In each of these rabbits very large numbers of readings of arterial pressure were made and the corresponding rates of infusion carefully measured   In Figs 7 and 8 each point represents the average of at least 10 determinations of arterial pressure made consecutively over a period of 5 minutes or more   The points obtained on the curve of increasing dosage (solid circles) are shown separately from those on the curve of decreasing dosage (open circles)   Fig 7 shows in a striking way what had been suspected from Fig 6, namely that the blood pressure rise in response to increasing doses of renin gradually

reaches a ceiling, and that with the highest dosages of 16 5 units the response is actually less than it was at 8 units per hour   Fig 8 shows a similar relationship except that the rate did not rise so high and no reduction in response was seen at the highest dosage   In Fig 7 points obtained on the curve of decrease in dosage lie among those obtained on the curve of increasing
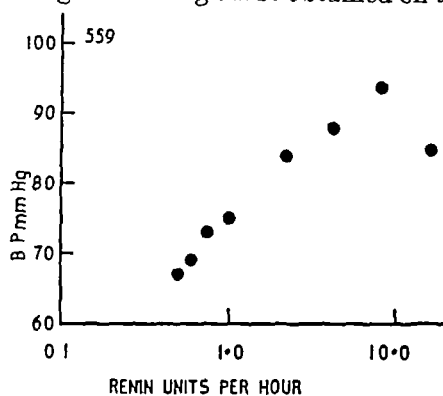

Fig 9   Rabbit 559   *Response plotted against dose on a logarithmic scale*

dosage   There is no difference in response when dose is increasing and when it is decreasing   In Fig 8 on the other hand all the points obtained on the downward curve of dosage lie significantly below those obtained on the upward curve, and the final base line lies below the initial   We cannot be sure of the explanation of this difference   It is not a property of a particular
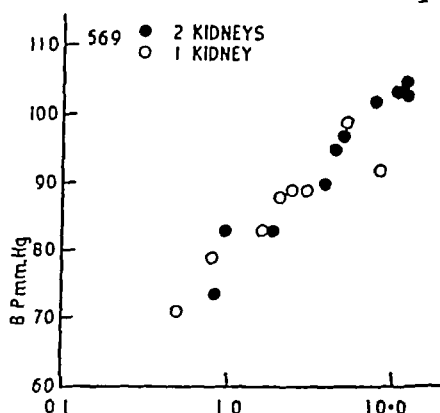

Fig 10   Rabbit 569   Response plotted against dose expressed on a logarithmic scale   The solid circles are the values obtained on the curve of ascending dosage, in the first experiment when the animal had two kidneys   The open circles are the values obtained on the curve of ascending dose in the second experiment after removal of a kidney

animal for in 569 one kidney was removed and the response to increasing and decreasing dosages again investigated   No difference between the ascending and descending curves was noted on this occasion   The pheno-menon of the lowering of the base line after renin infusions will be discussed later (p 236)

In Fig 9 and 10, the arterial pressure is plotted against dose expressed on a logarithmic scale In these figures the points have been obtained from the curves of increasing dosage in Figs 7 and 8, by averaging all the values for arterial pressure corresponding to a given dose Thus each of the points in Figs 9 and 10 represents at least 20, and usually upwards of 50 readings of arterial pressure By taking these large numbers we have hoped to eliminate some unavoidable factors which might, by their variable effects on arterial pressure, tend to obscure any relationship between dose and response In Fig 9 the relationship between response and the dose plotted logarithmically is essentially linear, with the striking exception of the value for the highest dose In Fig 10 the relationship is also linear This figure also contains the points similarly obtained for the second experiment on the same rabbit after unilateral nephrectomy The same linear arrangement is evident in the second experiment, with the possible exception of the values for the highest dose then used
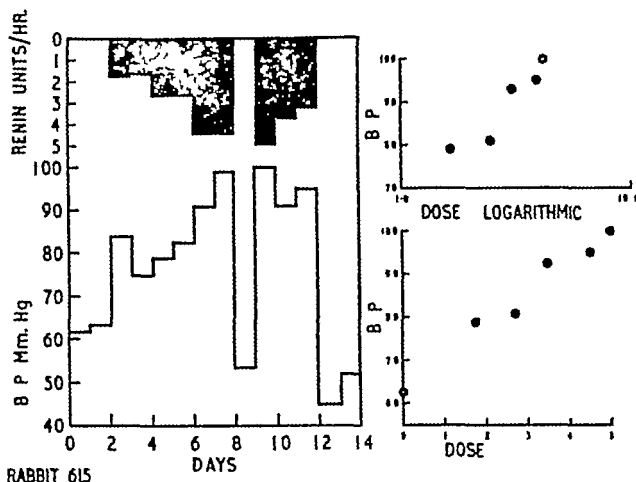

Fig 11   Summarises the data in rabbit 615   In this rabbit the renin was prepared differently
The hypertension is maintained, but the form of the dose response curve is not clear, possibly
due to the small range investigated or to the change in base line following the infusion.

In 5 other animals, 561, 562, 566, 567 and 599, the relationship between dose and response was investigated carefully though not quite as fully as in the two animals cited, and was found also to approximate to a logarithmic curve All the renin preparations used in these experiments were made by the alcohol method In the other experiments the relationship between dose and response has been less definite In some of these, the observations on arterial pressure were not numerous enough to exclude the influence of extraneous factors, but in others it seems extremely probable that a shifting base line may provide the explanation Fig 11 records such an experiment using a preparation made by the heating method Renin infusion was interrupted twice, the first time accidentally because the rabbit bit through

its catheter and, finally, by intention   The base line was progressively depressed on each occasion   It will be seen that it is questionable here whether the dose response curve is linear or logarithmic, a result that might be anticipated with a base line that is so variable

There have been very few previous studies of the relationship between the dose of renin and the response   The work reported here is in agreement with that of Pickering and Prinzmetal (24) who found that in the unanæsthetised rabbit the rise of pressure after a single intravenous injection was proportional to the logarithm of the dose   On the other hand Swingle and others (30) found a linear relationship between dose and response in the anæsthetised dog, but they investigated only a small range of dosage

The effect of terminating the infusion of renin   In all the experiments except two, renin was replaced by saline for one or two days and a second base line obtained   From Table I it may be seen that the base line after renin was never higher than it had been before   In 7 experiments the second base line was within 5 mm of the first, in 2 within 5-9 mm and in 2 within 10-20 mm , in 2 it was more than 20 mm lower than the first   Some of this fall in base line may be attributed to the circumstances of the experiment, perhaps to the animal becoming accustomed to its surroundings   But in the four control experiments using saline alone, the average of the readings obtained in the last 2 days, though always lower than the average of the first 2 days, never differed by more than 6 mm Hg   The large differences therefore seem to have been due to the effects of renin   The three largest falls occurred with two preparations of renin, and two of the three in animals whose kidney substance had been reduced   Nevertheless we cannot at present attribute this fall to any single proven factor   After infusions of synthetic l-adrenaline and d-l noradrenaline, the fall of the

TABLE III

Shows the rate at which the pressure falls to its base line after terminating renin infusions

Rabbit	Duration of hyper tension days	At end of renin infusion		After renin terminated			
		Rate renin units/hour	B.P mm.Hg	Lowest B.P reached mm.Hg	Base line mm.Hg	Time to fall to base hr	Base line before renin mm.Hg
561	17	5	101	33	57	1½	57
569	15	1 0	80	43	57	3½	61
554	7	1 8	83	60	63	3	69
562	18	2 1	98	64	64	4	68
559	13	0 8	73	53	55	5 to 20	57
585	6	2·0	96	56	57	2	78

base line of arterial pressure is more striking and more constant than after renin, and seems to be due to the release of a vasodilator substance or substances from the tissues in response to the prolonged action of the vasoconstrictor drugs   The same explanation may hold for renin, but the inconstancy of the phenomenon makes interpretation difficult

The time relations of the fall of pressure after stopping renin have been carefully investigated in 6 experiments, the results of which are summarised in Table III, and illustrated in Fig 12   In all these animals the arterial pressure began to decline soon after stopping the renin and had reached the final base line in from 1½ to over 5 hours   In two animals the pressure fell to a very low value and subsequently rose to level off at its final base line This phenomenon is not peculiar to renin, and has been frequently seen after adrenaline and noradrenaline infusions, where the fall of pressure is more rapid   It is evidently due to a vasodilator mechanism evoked by the pressor substance and persisting after it, but whether this is nervous or chemical we do not know
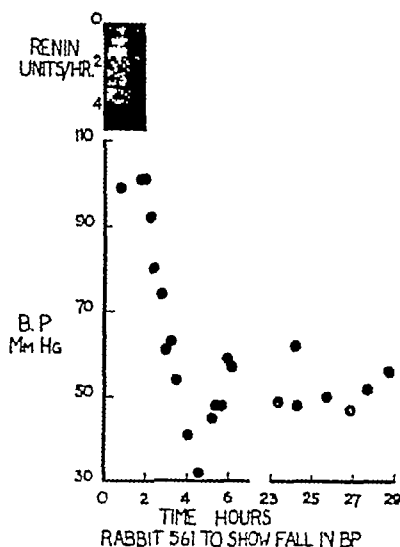

Fig 12   Shows the fall of arterial pressure after terminating the infusion in rabbit 561 (Fig 4) The time scale is interrupted between 6 and 23 hours

Papers from this laboratory have previously drawn attention to the similarity in the rate at which the pressure falls to normal after stopping an infusion of renin lasting 4 hours and after removing a single remaining kidney, ischæmic for one week   The observations here described provide more suitable data for this comparison, and show very much the same rate of decline of arterial pressure as did the shorter infusions   It has also been previously observed that when the sole kidney has been ischæmic for periods

of 2 months or more, its removal is not followed by a decline in arterial pressure within 3 days   Unfortunately we have not maintained renin infusions for this length of time and can only report that after our longest infusions of 17 and 18 days duration, the time taken for the arterial pressure to reach its base line, was not prolonged

The pulse rate during renin hypertension   Neither renin nor hypertensin seem to have any conspicuous action on the isolated heart (21, 32)   It would be anticipated therefore that the hypertension induced by renin would stimulate the carotid sinus and depressor reflexes with resultant slowing of the heart and vasodilatation   With a single intravenous injection of
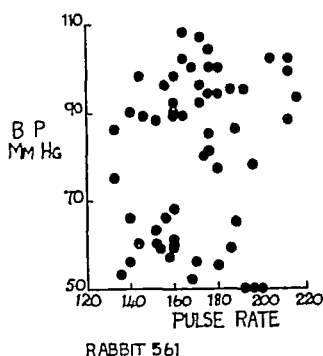

Fig 13   Summarises the simultaneous readings of pulse rate and blood pressure during renin infusion in rabbit 561

renin or hypertensin, conspicuous slowing of the heart is seen   Fig 13 shows the pulse rates and their corresponding blood pressure observed during an infusion of saline for 5 days and renin for 17 days   As will be seen there is no relationship between pulse rate and blood pressure   It is evident that in these experiments the heart rate was chiefly influenced by factors other than the arterial pressure

The response to a single intravenous injection of renin during the infusion Taggart and Drury (31) found that when hypertension was produced by a short infusion of renin, the response to a single injection of renin was reduced They related this to the dose response curve described by Pickering and Prinzmetal (24)   We have seen that during long infusions, response is related to the logarithm of the dose , and during these infusions reduction in the response to a single injection of renin is also to be expected   The response has been tested in 7 animals and the results are shown in Fig 14 The renin used for testing was a crude preparation made by extracting 1 g of our standard powder with 10 ml saline   ½ ml was injected into a vein of the denervated ear and the response measured during the initial and final saline infusion and during the renin infusion itself   It will be seen that the response to renin is reduced during the hypertension

This reduction is precisely what would be expected from the dose response curve But it is important that it should be established, for as Taggart and Drury pointed out and others have confirmed, the response to renin is not diminished in the animal with hypertension produced by constriction of the renal arteries, except in the early stages, and then not greatly (23)
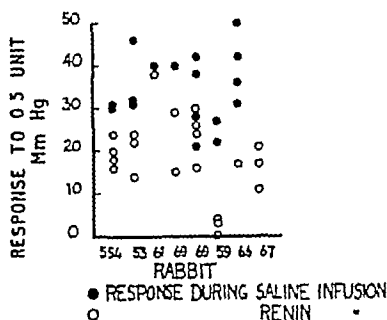

Fig 14   Summarises the responses to intravenous injection of 0 5 unit renin in 8 rabbits during saline and renin infusions

The effect of reducing the kidney substance   One rabbit, 569, was given an infusion of saline for 2 days, renin for 15 days and saline for 5 days, after which the catheter was removed and the right kidney was excised under nembutal anæsthesia   A week later the catheter was inserted in the other jugular vein and an infusion was given of saline for 2 days, renin for 14 days, and saline for one day   As already mentioned, in the first experiment on this rabbit, the responses to a given dose of renin were larger on ascending than on descending dosage   In the second experiment no such difference was observed   Fig 10 compares the responses on the curve of ascending doses in the two experiments   They are identical within the limits of variation   We may therefore conclude that removing one kidney does not alter the rabbits' behaviour to the continuous entry of renin into the circulation

In 4 animals the right kidney was removed, and 10 days or more later about one-third of the remaining kidney was amputated, hæmorrhage from the cut surface being arrested by a seaweed preparation and light retaining stitches   This procedure was usually followed by a transient hypertension and rise of blood urea lasting up to 6 weeks   Renin infusions were begun 8 days (567), 22 days (566), 28 days (583) and 31 days (585) after the renal amputation   Table IV compares the responses found in these rabbits with those obtained in intact rabbits   No systematic difference is apparent , there may be some tendency for the rabbits with reduced kidney substance to be less sensitive than those with normal kidneys

TABLE IV

Compares the rises of pressure (in mm Hg) above the initial base line produced by different rates of renin infusion in rabbits with normal and grossly reduced renal substance

Renin dose	Rise of pressure above base line in mm.Hg							—
	Animals with 2 kidneys				Animals with ⅔ kidney			
units/hr	559	569	561	562	566	567	583*	585*
0 – 0 5	10	—	—	—	—	—	—	—
0 5 – 0 75	12	15	—	—	—	—	—	—
0 75– 1 0	17	24	23	17	19	—	—	—
1 0 – 1 5	—	—	33	21	—	11	19	21
1 5 – 2 0	—	24	35	28	—	11	—	22
2 0 – 2 5	27	—	34	29	—	22	—	29
2 5 – 3 0	—	—	—	—	—	—	—	—
3 0 – 4 0	—	—	—	—	37	32	24	—
4 0 – 6 0	31	36	42	26	37	—	—	—
6 0 – 8 0	—	43	—	—	—	29	24	—
8 0 –10 0	37	—	—	36	—	30	—	—
10 0 plus	28	45	—	—	—	38	—	—
Blood urea—								
At beginning	58	41	36	34	70	47	43	32
At end	45	61	55	42	155	186	50	46

* Blood pressure readings less numerous and figure less reliable

It has been known since the observations of Tigerstedt and Bergman (32) that nephrectomy increases the response to renin Houssay, Braun-Menendez and Dexter (15) found that the survival of injected renin in the circulating blood was progressively greater with the lapse of time following nephrectomy Govaerts (9) observed in the nephrectomised dog a similar progressive increase in pressor response to the blood from another dog's kidney interposed in cross circulation It seems probable therefore that increased sensitivity to renin develops only gradually after complete nephrectomy Our finding that reduction of renal substance to the point of producing hypertension and urea retention does not increase the sensitivity to infusions of renin is thus not in conflict with earlier facts

It has been alleged that a normal kidney protects an animal against the hypertension resulting from renal ischæmia and that this is due either to the kidney secreting an antagonist to, or itself destroying, the hypertensive

secretion of its ischæmic fellow This idea which is fully discussed by
Braun-Menendez and others (3) is however meagrely supported by evidence
It rests chiefly on the fact that when one renal artery is constricted, removing
the other kidney increases the hypertension   As pointed out in an earlier
publication (25), this fact is equally open to the interpretation that removing
the normal kidney alters the ratio between blood supply and demand in its
ischæmic fellow and so increases the stimulus to the release of the hypertensive
agent

Discussion

Before discussing the implications of this work two possible sources of
error must be noted   The most important is that our renin was not chemically
pure We refined our renin by two very different methods in the hope that if
our results were in any way altered by an impurity, this might be demon-
strated No such discrepancy was found and in fact nothing in our experi-
ments has led us to believe that the results we have described in this paper are
due to anything but renin * Nevertheless the experiments should be repeated
with pure renin where this is available The second possible source of error is
the measurement of arterial pressure When precautions are taken, as they
were here, to keep the ear artery dilated, Grant and Rothschild (11) showed
that the pressure recorded by their capsule closely follows mean femoral
artery pressure , this was confirmed and extended to the rise of pressure
produced by renin (25), and we have extended it also to the hypertension
produced by noradrenaline infusions (1) The arterial pressures recorded
in this paper thus probably approximate closely to mean femoral artery
pressure, and should demonstrate any changes in this value

The results described are of chief interest in relation to the general
function of renin in the body and its possible role in hypertension Renin,
or its precursor, is a normal constituent of the renal cortex and seems to be
released into the renal vein blood when the systemic arterial pressure falls
(16, 28) It is evident from the experiments described in this paper that
its release into the blood at a constant rate over a period of many days
produces a relatively constant and stable alteration of the circulation ,
its release at increasing rates intensifies the changes, but in so far as the
blood pressure reflects them, to a progressively diminishing degree   In
the constancy of the circulatory change over long periods, renin contrasts
sharply with noradrenaline and perhaps adrenaline

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* We have wondered whether the lowering of the final, as compared with the initial, base line
which occurs in only some experiments may be due to the effect of impurities that are present to a
significant extent in some renin preparations but not in others   This explanation may prove to
be correct, but is open to the objection that an even more constant fall of final base line is seen
when infusions of pure adrenaline and noradrenaline are given.

TABLE IV

Compares the rises of pressure (in mm Hg) above the initial base line produced by different rates of renin infusion in rabbits with normal and grossly reduced renal substance

| Renin dose units/hr | Rise of pressure above base line in mm.Hg | | | | | | | |
| | Animals with 2 kidneys | | | | Animals with ¾ kidney | | | |
	559	569	561	562	566	567	583*	585*
0 – 0 5	10	—	—	—	—	—	—	—
0 5 – 0 75	12	15	—	—	—	—	—	—
0 75– 1 0	17	24	23	17	19	—	—	—
1 0 – 1 5	—	—	33	21	—	11	19	21
1 5 – 2 0	—	24	35	28	—	11	—	22
2 0 – 2 5	27	—	34	29	—	22	—	29
2 5 – 3 0	—	—	—	—	—	—	—	—
3 0 – 4 0	—	—	—	—	37	32	24	—
4 0 – 6 0	31	36	42	26	37	—	—	—
6 0 – 8 0	—	43	—	—	—	29	24	—
8 0 –10 0	37	—	—	36	—	30	—	—
10 0 plus	28	45	—	—	—	38	—	—
Blood urea— At beginning	58	41	36	34	70	47	43	32
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It has been alleged that a normal kidney protects an animal against the hypertension resulting from renal ischæmia and that this is due either to the kidney secreting an antagonist to, or itself destroying, the hypertensive

secretion of its ischæmic fellow   This idea which is fully discussed by Braun-Menendez and others (3) is however meagrely supported by evidence   It rests chiefly on the fact that when one renal artery is constricted, removing the other kidney increases the hypertension   As pointed out in an earlier publication (25), this fact is equally open to the interpretation that removing the normal kidney alters the ratio between blood supply and demand in its ischæmic fellow and so increases the stimulus to the release of the hypertensive agent

Discussion

Before discussing the implications of this work two possible sources of error must be noted   The most important is that our renin was not chemically pure   We refined our renin by two very different methods in the hope that if our results were in any way altered by an impurity, this might be demonstrated   No such discrepancy was found and in fact nothing in our experiments has led us to believe that the results we have described in this paper are due to anything but renin *   Nevertheless the experiments should be repeated with pure renin where this is available   The second possible source of error is the measurement of arterial pressure   When precautions are taken, as they were here, to keep the ear artery dilated, Grant and Rothschild (11) showed that the pressure recorded by their capsule closely follows mean femoral artery pressure , this was confirmed and extended to the rise of pressure produced by renin (25), and we have extended it also to the hypertension produced by noradrenaline infusions (1)   The arterial pressures recorded in this paper thus probably approximate closely to mean femoral artery pressure, and should demonstrate any changes in this value

The results described are of chief interest in relation to the general function of renin in the body and its possible role in hypertension   Renin, or its precursor, is a normal constituent of the renal cortex and seems to be released into the renal vein blood when the systemic arterial pressure falls (16, 28)   It is evident from the experiments described in this paper that its release into the blood at a constant rate over a period of many days produces a relatively constant and stable alteration of the circulation , its release at increasing rates intensifies the changes, but in so far as the blood pressure reflects them, to a progressively diminishing degree   In the constancy of the circulatory change over long periods, renin contrasts sharply with noradrenaline and perhaps adrenaline

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* We have wondered whether the lowering of the final, as compared with the initial base line which occurs in only some experiments may be due to the effect of impurities that are present to a significant extent in some renin preparations but not in others   This explanation may prove to be correct, but is open to the objection that an even more constant fall of final base line is seen when infusions of pure adrenaline and noradrenaline are given.

These observations also show that the hypothesis that a maintained hypertension can be produced by the constant release of renin into a vein is perfectly tenable   It is to be noted that our animals looked well, behaved normally, showed no disturbance in any bodily function obvious to the eye, and showed no macroscopic abnormalities when dissected after death They also resembled animals with renal artery hypertension in the normal macroscopic appearance of the ear vessels

One of Taggart and Drury's (31) main criticisms of the renin hypothesis was that in their rabbits infusion of renin failed to raise the arterial pressure by more than 30 mm Hg , while renal artery constriction could take it 40 mm higher   We too have been impressed with this difference   In none of our intact animals were we able to raise the arterial pressure by more than 40-50 mm Hg by infusion of renin   The maximum rise was remarkably constant in spite of variations in the response to a single small dose   Intact animals are, however, not necessarily comparable to animals in which one kidney has been removed and the function of the other disturbed by constricting the renal artery   It was for this reason that we investigated the effect on the response to infused renin of reducing kidney substance to a degree that would produce hypertension and urea retention   As has been

TABLE V

Distribution of hypertension in rabbits with constriction of the renal artery of different duration

Rise of pressure mm.Hg	Hypertension lasting up to 2 weeks	Hypertension lasting 2 months or more
0–19	4	2
20–29	1	3
30–39	6	4
40–49	1	5
50–59		4
60–69		3
70–79		1
Largest rise	40 mm	73 mm

seen no alteration in the response to infused renin was found, though the initial base line was raised   There is, however, an even more important point to be made in any comparison of this kind   Previous work from this laboratory has stressed the importance of insuring that when two forms of hypertension are compared, they are comparable in duration (23)   Table V shows the rises of blood pressure produced by constricting the renal artery or

arteries (a) within the first two weeks and (b) after 2 months or more   This
Table comprises the data from all the relevant animals contained in the
Tables of two earlier publications (23, 26)   It will be seen that the degree of
hypertension found in the rabbit with renal artery constriction of up to two
weeks duration can be easily matched by renin infusions of the same duration
To match the higher levels infusions at a rate of 4 units per hour would be
required   Pickering, Prinzmetal and Kelsall (26) found that the average
renin content of the sole ischæmic kidney in 8 animals with hypertension of 2
to 8 days duration was 32 units   It is clear therefore that to produce the
order of hypertension observed the whole renin content of the kidney would
need to be discharged and reformed about once every 8 hours, by no means
an impossibility

If we accept the diodone clearance as measuring renal plasma flow,
and assume that the blood flow of a single remaining kidney with artery
clamped is about half of that of a pair of normal kidneys, then we find that
4 units of renin would be released in one hour into about 1½ litres of blood *
It is the present practice to look for renin in renal venous blood   If all the
renin released into the renal vein is destroyed by the time it gets back to the
kidney, then the concentration of renin should be of the order of 0 0027
units per ml in the renal vein blood   It is doubtful whether many of the
methods of assay at present available would detect this   If on the other
hand the renin is not destroyed so quickly in blood, and the evidence is in
this sense (15, 26), it may accumulate to much higher levels , but if so there
seems little point in obtaining renal vein blood for assay, particularly when
as Govaerts and Vermory (10) have pointed out altering the circulatory
condition in the kidney may itself favour the release of renin

The last point to notice in comparing renin and renal artery hypertension
is that the times taken for the arterial pressure to regain its base line after
excising the sole ischæmic kidney and after stopping an infusion of renin are
similar, provided the hypertension has lasted less than 14 days   When,
however, the levels of hypertension reached with renal artery constriction
lasting 2 months or more are considered, it is clear that the figures are
of a different order from those produced by infusions of renin lasting up
to 18 days   In this sense our conclusions agree with those of Taggart
and Drury (31)   It is evident that some new factor has intervened in
this chronic hypertension, and, while cardiovascular hypertrophy may
have altered the sensitivity to renin and thus the levels of hypertension
reached, this will not account for the failure of the arterial pressure to fall
when the sole ischæmic kidney is excised   In 1938 Hessel (13) reported
briefly some experiments on rabbits which, it seemed, might throw light on
this phenomenon   He stated that when 4 to 6 strongly pressor doses of

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* A representative figure for diodone clearance is 35 ml. per min. (17), and for hæmatocrit
30%, giving a renal blood flow of 50 ml per min or 1½ litres per hour

renin, or an intravenous infusion, lasting several hours, were given daily, the arterial pressure eventually remained high 12 hours after the last renin injection and finally remained raised for months after ceasing injections entirely In the example cited the animal had a pressure of 75 mm during the preliminary period, 90 mm after the first and 105 mm after the fifth and final week of injections , its pressure remained between 110 and 125 mm Hg in the subsequent 7 months Our experiments have not lasted so long, but even after 18 days continuous infusion no tendency was observed for the pressure to be raised after the renin was discontinued

Summary

1 A method has been devised for infusing sterile solutions of known composition into the jugular vein of the unanæsthetised rabbit for periods up to 23 days

2 Provided asepsis is absolute, continuous infusions of renin lasting up to 18 days produce hypertension that is maintained, and the animals remain in good condition

3 Within the lower range of dosage tested, a constant intravenous dose of renin produces a relatively constant hypertension at least for periods of the order of 10 days

4 Equal increments in the dose of renin produce progressively smaller increments in the degree of hypertension With the highest rates of dosage tested, the degree of hypertension tends to fall and the response to injected renin is suppressed

5 The relationship between the rise of arterial pressure and the logarithm of the dose is approximately linear, except with very high doses

6 No alteration was detected in the response to renin infusions by removing one and one third kidneys

7 The degree of hypertension produced in the rabbit by renal artery constriction lasting up to 2 weeks is comparable with that produced by renin infusions of similar duration Hypertension comparable to that produced by renal artery constriction lasting 2 months or more was not obtained by renin infusions of much shorter duration

8 The times taken for the arterial pressure to regain its base line after excising the sole ischæmic kidney and after stopping a renin infusion are similar provided the duration of hypertension is of the order of 2 weeks

9 The observations are compatible with the hypothesis that hypertension following renal artery constriction is due to the release of renin into the renal vein at least as far as the first 2 weeks are concerned No light is thrown on the problem of chronic hypertension

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THE EFFECTS OF PROLONGED INFUSIONS OF NORADRENALINE AND ADRENALINE ON THE ARTERIAL PRESSURE OF THE RABBIT

By R B BLACKET,* G W PICKERING and G M WILSON

BAINBRIDGE and Trevan (1) found that infusion of adrenaline into the anæsthetised dog produced a hypertension that was only transient and was followed by a depression of arterial pressure and circulatory failure   This failure to produce maintained hypertension with adrenaline has been confirmed by other workers (4, 5), and particularly by Freeman and others (6) in unanæsthetised dogs

Recently it has become evident that noradrenaline is probably as important as adrenaline in the physiology of the circulation (2)   Moreover its constrictor rather than dilator action on muscle, its relatively slight effect on cardiac output (8) and the poverty of symptoms when it is infused intravenously in man (2) make its role in hypertension much more likely

A method has recently been described from this laboratory (3) for infusing sterile solutions at a known rate into the external jugular vein of the unanæsthetised rabbit   It has been shown that saline infusions at the rate of 4 ml per hour lasting up to 14 days produce no constant or significant trend in arterial pressure   Renin in suitable doses produces a rise of pressure that is maintained up to 18 days, the longest infusion investigated   In this paper we describe experiments to test whether noradrenaline and adrenaline would also produce a maintained hypertension, a question particularly relevant to the maintained hypertension sometimes seen in phæochromocytoma

Method

The method used has been fully described in a previous paper (3)   The infusions were made from 100 ml burettes into a ureteric gum elastic catheter inserted, under nembutal anæsthesia, in the external jugular vein of brown male rabbits weighing 2 to 2 5 kg   After recovery from anæsthesia saline was infused for at least 2 days and followed by the adrenaline or noradrenaline solution to be tested   The stock adrenaline and noradrenaline solutions from which the dilutions in the burette were made were 1 1,000 solutions prepared according to the B P formula,†

autoclaved and kept in the refrigerator in the dark   Strict asepsis was maintained throughout and was controlled by taking cultures from the burettes daily at the end of each day's run   To each burette was added 250,000 units of penicillin, and (excepting the first), 1,000 units of heparin The infusion rate was maintained at about 4 ml per hour, so that the 100 ml burettes needed changing about once daily   Changes in dosage were made by varying the concentration of adrenaline or noradrenaline in the burette   The animals were fed thrice daily with oats, bran and greenstuff, were given water from a bottle, and their cages were cleaned out daily Hæmoglobin estimations were made on ear blood by a photo-electric method For the blood urea determinations we are indebted to Dr Roche Lynch and his staff

Results

Measurement of arterial pressure   The arterial pressure was measured in the ear of the warm animal by Grant and Rothschild's capsule (7)   This method has been shown to give readings that agree well with mean femoral artery pressures in the anæsthetised warm animal and during the rise of pressure produced by renin (12)   Renin, however, has little effect on the size of the central artery of the ear, whereas noradrenaline produces not only a paling of the ground substance but a clear decrease in the diameter of the central artery   It was first necessary, therefore, to see whether reliable pressures could be obtained from the ear artery under the action of noradrenaline

Under nembutal anæsthesia, the femoral artery of a rabbit was canulated and the arterial pressure recorded by a mercury manometer in the usual way   An infusion was given into an ear vein on one side and the arterial pressure measured by Grant and Rothschild's capsule on the central artery of the other ear   The animal was kept warm by a hot pad, so that the ears remained flushed throughout   Infusions of noradrenaline of various strengths were given at varying rates to produce widely varying levels of arterial pressure

Simultaneous readings of arterial pressure from the femoral artery manometer and from the ear capsule are plotted against one another in Fig 1   It will be seen that there is a fair agreement between the two values throughout the range tested, and it may be concluded that the capsule does give a valid reading of arterial pressure even when a considerable hypertension has been produced by noradrenaline

Effects of infusions of noradrenaline   Infusions of d-l noradrenaline were given in 4 animals after base lines for blood pressure and pulse had been obtained for 3 to 6 days during infusions of saline   The results are summarised in Table I and illustrated by Figs 2 and 3   Cultures from the burettes in all these animals remained sterile

It will be seen from the Table that maintained hypertension was produced in one animal only, 590   In this animal after three days on saline, noradrenaline was begun at the rate of 1 6µg per min which raised the pressure from an average of 59 to 76 mm on the first day   The highest pressure reached was 88 mm on the 5th day of noradrenaline with a dose of 4 5µg per min   Thereafter the pressure declined   On the 7th day (8 5µg per min) it was difficult and on the 8th day (8µg per min) impossible to get readings of arterial pressure owing to vasoconstriction in the ear , the animal was listless and not eating   Noradrenaline was stopped and
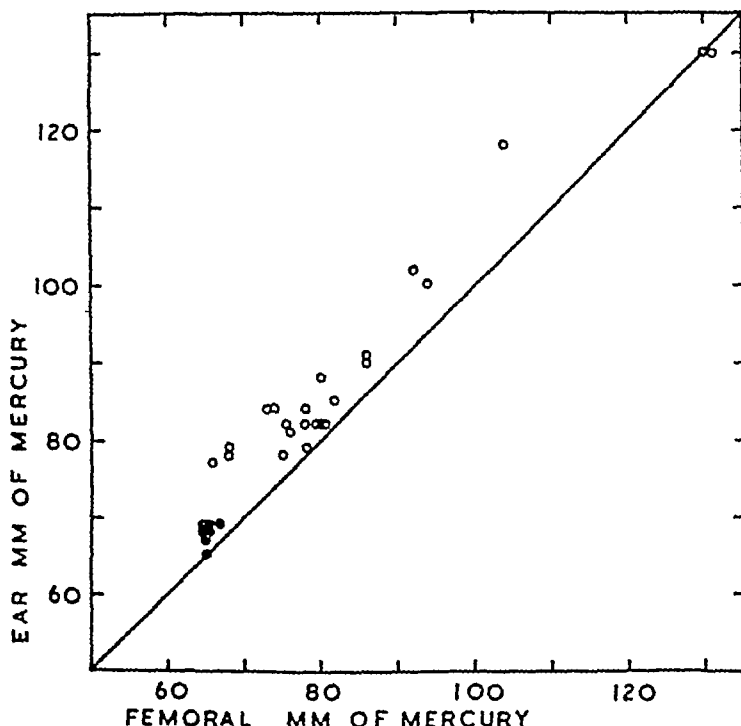

Fig 1   Rabbit anæsthetised with nembutal   Infusion of noradrenaline left ear   To show the readings of arterial pressure recorded simultaneously by the capsule from the central artery of the flushed right ear, and by a mercury manometer from the femoral artery   Closed circles readings before, open circles readings during, infusion of noradrenaline

saline was begun, but for the two days during which saline was infused, the animal remained too ill for readings to be obtained   Postmortem examination showed enormous dilatation of the large gut with much gas and semi-fluid fæces   The three remaining animals developed less sustained and less intense hypertensions   All three became ill during the infusions , they were listless, did not eat and neglected their fur   Rabbit 588 was found dead the day after the noradrenaline was stopped because of illness Rabbit 591 recovered after saline infusion, and a second base line was

TABLE 1

Summarises the data from animals receiving continuous infusions of adrenaline and noradrenaline The initial and final base lines represent the average pressures for at least two days preceding and succeeding the adrenaline or noradrenaline and during which the animal received saline alone

Animal	Substance	Range of dose µg per min	Base line		Days B P raised over initial base line mm Hg				Total infusion adrenaline or noradrenaline Days
			Initial	Final	<0	1-10	11-20	21-30	
500*	d l Noradrenaline	1 5 to 8 5	59	—	0	0	6	2	8
501*	d l Noradrenaline	4 5 to 12 0	63	47	0	2	2	1	5
588†	d l Noradrenaline	5 0 to 13 8	61	—	2	3	2	1	8
014	d l Noradrenaline	1 0 to 12 6	71	52	5	4	0	0	9
010	l Adrenaline	1 0 to 1 0	66	55	0	4	0	0	4
007	l Adrenaline	0 15 to 0 72	68	46	5	1	0	0	6
	d l Noradrenaline	1 5 to 7 2							
012	l Adrenaline	0 1 to 0 5	63	57	2	1	2	1	6
	d l Noradrenaline	1 0 to 5 0							

* Rabbit became ill, drug discontinued
† Rabbit became ill, and died during infusion

obtained   Both these animals showed gross dilatation of the terminal large gut postmortem, the appearances resembling those in 590   Instead of the interrupted series of dry pellets with empty gut in between, the bowel contained an irregular collection of small wet pellets with some mucous and there was great distension with gas   Although we did not weigh the fæces it was our impression that they were reduced in total amount during the period of noradrenaline infusion   They were certainly more fluid than
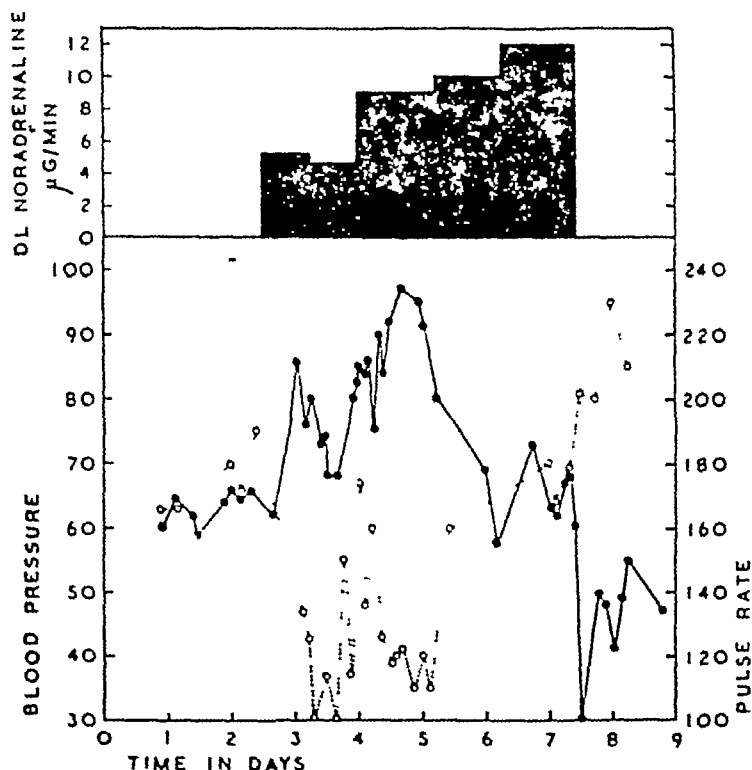

Fig 2   Rabbit 591   Shows the course of arterial pressure (solid circles) and pulse rate (open circles) during an infusion of d l noradrenaline   During the period before and after noradrenaline the animal received saline at the same rate

normal and decreased in bulk   Thus in normal rabbits the average volume of a pellet was 0 35 ml , in 614 on a dose of 10 µg noradrenaline per min the average volume was 0 26 ml

Figs 2 and 3 show the behaviour of pulse rate and blood pressure in Rabbits 591 and 614   Both of these animals had an initial rise of pressure that was not maintained   Both show very clearly that noradrenaline hypertension is accompanied by bradycardia and that a profound depression of arterial pressure supervenes within a few minutes of terminating the infusion

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Summarises the data from animals receiving continuous infusions of adrenaline and noradrenaline   The initial and final base lines represent the average pressures for at least two days preceding and succeeding the adrenaline or noradrenaline and during which the animal received saline alone

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588†	d l Noradrenaline	5 0 to 13 8	61	—	2	3	2	1	8
014	d l Noradrenaline	1 0 to 12 6	71	52	5	4	0	0	9
010	l Adrenaline	1 0 to 1 0	66	55	0	4	0	0	4
'007	l Adrenaline / d l Noradrenaline	0 15 to 0 72 / 1 5 to 7 2	68	46	5	1	0	0	6
'012	l Adrenaline / d l Noradrenaline	0 1 to 0 5 / 1 0 to 5 0	63	57	2	1	2	1	6

* Rabbit became ill, drug discontinued
† Rabbit became ill, and died during infusion

obtained   Both these animals showed gross dilatation of the terminal large
gut postmortem, the appearances resembling those in 590   Instead of the
interrupted series of dry pellets with empty gut in between, the bowel
contained an irregular collection of small wet pellets with some mucous
and there was great distension with gas   Although we did not weigh the
fæces it was our impression that they were reduced in total amount during
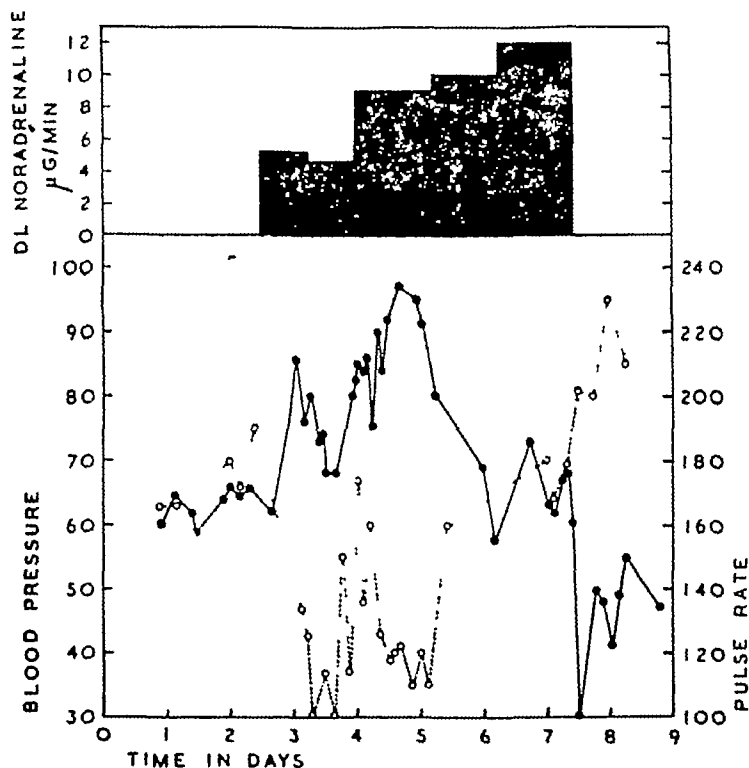the period of noradrenaline infusion   They were certainly more fluid than

Fig 2   Rabbit 591   Shows the course of arterial pressure (solid circles) and pulse rate (open
circles) during an infusion of d l noradrenaline   During the period before and after
noradrenaline the animal received saline at the same rate

normal and decreased in bulk   Thus in normal rabbits the average volume
of a pellet was 0 35 ml , in 614 on a dose of 10 μg noradrenaline per min
the average volume was 0 26 ml

Figs 2 and 3 show the behaviour of pulse rate and blood pressure in
Rabbits 591 and 614   Both of these animals had an initial rise of pressure
that was not maintained   Both show very clearly that noradrenaline
hypertension is accompanied by bradycardia, and that a profound depression
of arterial pressure supervenes within a few minutes of terminating the
infusion

In contrast to renin, noradrenaline produces a very definite paling of the ground tone of the ear and a diminution in size of the central artery when the animal is warm and would normally have flushed ears   The temperature of the ear in such circumstances is much reduced   We have made no microscopical investigations but we would suspect from this that the arteriolovenous anastomoses are closed by noradrenaline

Adrenaline   Only one animal received l-adrenaline, in doses of from 1 to 1 9 µg per min for 4 days   The greatest rise of arterial pressure was
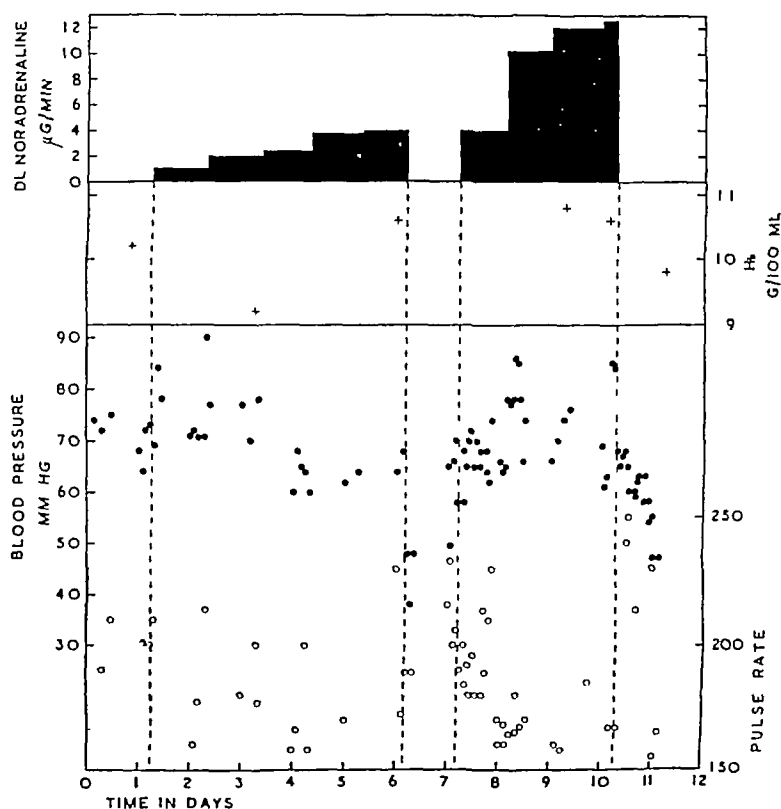
Fig 3   Rabbit 614   Shows arterial pressure (solid circles), pulse rate (open circles), and hæmoglobin (crosses) during infusion of saline and noradrenaline   The times and rate of the noradrenaline infusion are shown at the top of the figure

from a base line of 66 to 76 mm on the first day   Thereafter the pressure was very little above the base, though the appearance of the ear, identical with that described for noradrenaline and possibly more intense, indicated that the adrenaline was acting   The animal did not eat well during the second and third days of the infusion, and the fæcal pellets were small and wet   Bradycardia was inconspicuous but the pulse was irregular   Stopping the infusion was accompanied by a profound fall of arterial pressure to an average of 40, 57, and 53 mm on the three succeeding days

Adrenaline and noradrenaline together Since adrenaline and noradrenaline are present together in suprarenal medulla and in phæochromocytoma (10), and since phæochromocytoma may produce a continuous hypertension which has been attributed, perhaps erroneously, to the secretions of the tumour (2), we tried infusing a mixture choosing a ratio of 1 l-adrenaline to 5 l-noradrenaline (1 10 d-l noradrenaline), that is approximately the ratio found in phæochromocytoma Two animals were used In rabbit 612, the pressure rose from a base line of 63 to 83 mm on the 1st and 91 mm on the 2nd day, the dose of d-l noradrenaline being 1 1 and 2 5μg per min respectively, and of adrenaline 0 1 and 0 25μg per min There was no bradycardia On the same dose the pressure fell back
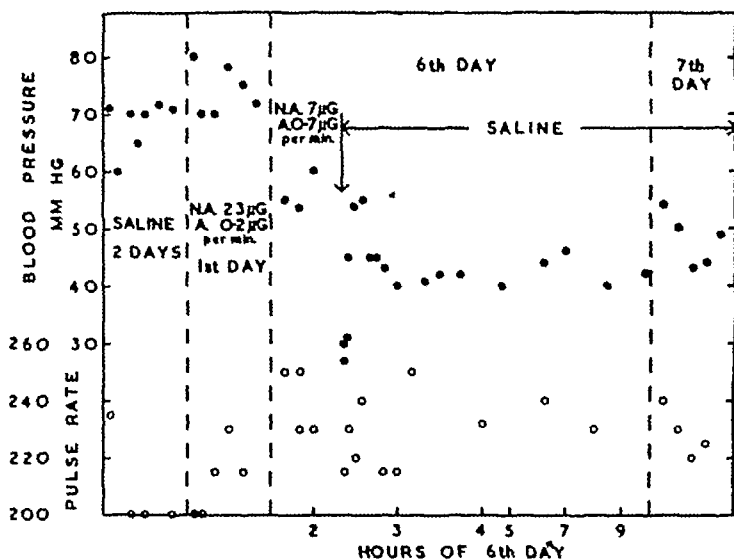
Fig 4 Rabbit 607 Shows the blood pressure (solid circles) and pulse rate following the termination of an infusion of noradrenaline and adrenaline The time scale is shown accurately for the day terminating the infusion, for the other days shown it is semi diagrammatic The animal received saline for 2 days then noradrenaline and adrenaline for 6 days (1st to 6 days), then saline for 24 hours

to the base in the next three days, rising slightly to 75 mm on 5 0 and 0 5μg per min of the substances on the 6th day On changing the infusion to saline the pressure fell abruptly to 46 mm and remained beneath its original base line on this and the succeeding days In rabbit 607 the rise of pressure was even less conspicuous , the fall on stopping the infusion is shown in detail in Fig 4 Both animals remained comparatively well during the infusion though both excreted small fæcal pellets whose volume in rabbit 612 averaged 0 25 ml In both animals the ears showed the changes in vascular calibre described for noradrenaline When the vasoconstrictor substances were replaced by saline the ears became flushed conspicuously and beyond the normal

DISCUSSION

So-called adrenaline " shock " was investigated repeatedly during and after the first world war Bainbridge and Trevan (1) injected adrenaline slowly into a vein in anæsthetised dogs for 20 min or longer at a rate sufficient to maintain the arterial pressure at a supra-normal level comparable with that due to stimulation of a sensory nerve The portal venous pressure rose to high levels, the systemic little if at all , the hæmoglobin rose, for example from 95 to 129% After stopping the adrenaline the arterial pressure fell to low levels while the portal pressure remained high and the liver turgid Erlanger and Gasser (4) injected 6 to 11 ml of 1 1,000 adrenaline in 21 to 90 min intravenously in dogs under ether anæsthesia With the largest doses the rise of arterial pressure was not maintained during the infusion After terminating the injection there was a great fall of pressure which slowly regained its original level after the small but not after the large infusions There was great hæmoconcentration They confirmed the rise of portal pressure but concluded it was not the cause of shock since blocking the portal radicles of the liver with lycopodium spores gave a similar rise in portal pressure but not the changes in arterial pressure Other workers confirmed the decrease in plasma volume in such experiments (5) and this became generally accepted as the basis of shock Freeman and his colleagues (6) repeated these experiments on unanæsthetised, trained dogs, injecting adrenaline at a rate of 0 0034 to 0 016 mg per kg per min for 1 to 1½ hours intravenously while the arterial pressure was recorded manometrically from a needle in the femoral artery They found a similar fall of arterial pressure beginning during the infusion and reaching low levels after it, accompanied by slowing of the peripheral blood flow and great hæmoconcentration The dogs frequently died with this picture of circulatory failure during the infusion or after it, but transfusion would restore the arterial pressure and keep the animals alive After death the chief changes were found in the gut , the intestines were soggy and the lumen contained blood stained fluid

In the experiments quoted, the amount of adrenaline given was outside the physiological range and was greater than the doses used in the observations here described The largest dose we used was 0 003 mg per kg per min of l-noradrenaline as compared with Freeman's 0 0164 mg per kg l-adrenaline (6) Nevertheless it seemed that the circulatory changes here found might also be due to loss of plasma from the circulation We accordingly estimated either the packed cell volume or the hæmoglobin in 5 animals , the results are summarised in Table II Significant hæmoconcentration was not found There was no evidence of the very slow blood flow observed by Freeman (6) and by Erlanger and Gasser (4) after terminating the infusion, since the ears of our animals were warm and red

A second possible explanation for the lack of sustained hypertension is inactivation of adrenaline and noradrenaline by oxidation or otherwise

during its exposure to room temperature for 24 hours in the burette   Two observations excluded this   When at the end of 24 hours the burette was replaced by a new one containing a solution of similar composition but freshly prepared, there was no further rise in arterial pressure   Furthermore even though the arterial pressure was not raised during infusions of noradrenaline and adrenaline the changes in the ear vessels indicated that in fact a vasoconstrictor substance was at work

A third possibility is the progressive intervention of the proprioceptive cardiovascular reflexes from the carotid sinus and aortic arch   The bradycardia which was so conspicuous with noradrenaline may be presumed

TABLE II

Estimations of hæmatocrit and hæmoglobin before, during and after infusions of adrenaline and noradrenaline, hæmatocrit in %, hæmoglobin in g per 100 ml

Rabbit	Before	During	After
588	31% 9 7 g 26% 8 2 g 27% 7 8 g 29% 7 2 g 31% 7 3 g 26% 7 6 g	29% 7 0 g 6 2 g	
612	7 4 g	7 1 g (2) 6 8 g (4)	7 2 g
614	10 2 g	9 2 g (2) 10 6 g (5) 10 8 g (8) 10 6 g (9)	9 8 g
610	9·0 g	9 6 g (3)	
607	9 0 g	8 6 g (3)	8 1 g 8 4 g

The figure in brackets after the hæmoglobin denotes the day of infusion.

to be so caused   Nevertheless inspection of the data shows that during the infusion, the fall of pressure is not associated with a further decrease in heart rate but may be associated with an increase   Moreover the conspicuous drop in pressure after discontinuing the drug can not be entirely attributed to the activity of these reflexes   As Fig 4 illustrates, the conspicuous fall of pressure may not be associated with an appreciable quickening of the heart

A fourth possibility is that adrenaline and noradrenaline were destroyed with increasing rapidity in the body as the infusion proceeded   This possibility is not consistent with the continued evidence of vasoconstriction seen in the ear of the warm animal , nor would it explain the profound fall

of arterial pressure after terminating the infusion of noradrenaline and adrenaline, unless, as indeed it might, the destructive mechanism also affects the chemical transmission of sympathetic impulses

The most likely explanation is that the long continued action of adrenaline and noradrenaline causes a release into the circulation of a vaso-dilator substance or substances, the release continuing for some time after the vasoconstrictor substances cease to act   This would account for the failure of hypertension to be maintained and for the profound and prolonged fall of arterial pressure subsequently   Adrenaline is a notable example of a vaso-active substance that affects the various vascular territories of the body in a conspicuously unequal manner   It produces a profound constriction of the skin, possibly of the gut, and increases muscle flow   Noradrenaline has a much less uneven action, but it has been less fully investigated   It may be therefore that tissues for long deprived of their customary blood supply release vasoactive substances into the circulation   But it is also conceivable that the vasodilator substances have a different origin

Green and others (9) have described a fall in arterial pressure in human subjects after terminating adrenaline infusions at the rate of 0 1 to 1 5 µg per min for 1½ hours   This fall was also unaccompanied by hæmo-concentration and was ascribed to vasodilatation

It is of some interest to contrast the results here obtained with infusions of noradrenaline and adrenaline and those obtained on infusing renin by a precisely similar method   With renin, provided infection of the solutions was avoided, the animals remained well, no obvious change in function of any organ occurred, the ear vessels appeared little altered macroscopically, the arterial pressure stayed high and in many animals was related to dose both on the curve of ascending and descending dosage   After discontinuing the renin, the pressure fell to the original base line in about half the experiments and to below it in the remainder   Thus the effects of renin differ from those of noradrenaline and adrenaline in three ways, namely there is no evidence from the skin of changed distribution of blood, hypertension is maintained , after terminating the infusion, the arterial pressure falls below the base line in only half the cases and in most of those relatively slightly   The experiments described in this and the previous paper thus suggest that renin merits very serious consideration as an agent that may produce maintained hypertension, but that it is still unproved that adrenaline and noradrenaline can act in this way

SUMMARY

1   Intravenous infusions of sterile solutions of d-l noradrenaline l-adrenaline and a mixture of the two have been maintained in rabbits for periods up to 8 days

2   With these infusions there was an initial rise of pressure that was in general not maintained despite an increasing dosage

3   Replacing the solutions of d-l noradrenaline, of l-adrenaline, or of the mixture, by saline was followed quickly by a profound fall of arterial pressure, which lasted in a lesser degree for several days

4   Significant hæmoconcentration was not found, but many of the animals appeared ill during the infusion   An alteration of the character of the fæces was usual and in animals receiving d-l noradrenaline the large gut was distended with gas at autopsy

5   It is suggested that the failure of hypertension to be maintained during, and the profound fall of pressure after, a prolonged infusion of noradrenaline and adrenaline is due to release of vasodilator substances into the circulation

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OBSERVATIONS ON THE DISTRIBUTION OF SUBCUTANEOUS FAT

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WHEN subjects gain or lose weight there is an obvious change in the body contours and in the thickness and texture of the subcutaneous tissues Sometimes these contours follow unusual but distinct patterns which have come to be associated with a particular disease process whilst other patterns have been described as distinct but not related to any clearly defined disorder   The contours of the body are determined by two factors   by those of the underlying muscle and bone, and by the thickness of the subcutaneous tissues from place to place   With an increase in weight which is not associated with growth there is little change in the contours of the carcase and the thickening subcutaneous tissue becomes the predominant cause of the body outline , gaps between muscles become filled in, bony points appear less prominent and the shape of the body becomes increasingly determined by the pattern of increase in thickness of the subcutaneous tissue   It is difficult to estimate the quantity of muscle in the living subject, but it is possible to estimate the thickness of the subcutaneous tissues by a relatively simple and rapid method   This paper describes such a method and the evidence obtained about the way in which the subcutaneous tissue contributes to the changing contours of a normal person who is gaining or losing weight

Method

Briefly the method consisted in measuring the thickness of the subcutaneous tissue fold at a large number of anatomically determined points   The method was used in a series of female subjects with different degrees of obesity

The method of estimating subcutaneous tissue fat by measuring the thickness of the skin and subcutaneous tissue fold with calipers has been in use for some time as an index of the state of nutrition and in somatotyping, but most workers have used only one or two points on the body for their measurements   It was appreciated that the method was crude and inaccurate but it was hoped that by taking a large number of measurements,

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some of the random sampling errors would be reduced in the estimation of total fat   In order to get an idea of the pattern of distribution of subcutaneous fat it was also necessary to use a sufficient number of points to cover the major variations in thickness over the body

The choice of sites was determined by several factors   The ease with which a fold of skin may be raised depends upon the looseness of the areolar attachment of the superficial to the deep fascia and on the texture of the subcutaneous tissue itself   The subcutaneous tissue over the buttock is too fibrous and too firmly attached to the deep fascia for satisfactory measurement and in the neighbourhood of skin creases the mobility of the
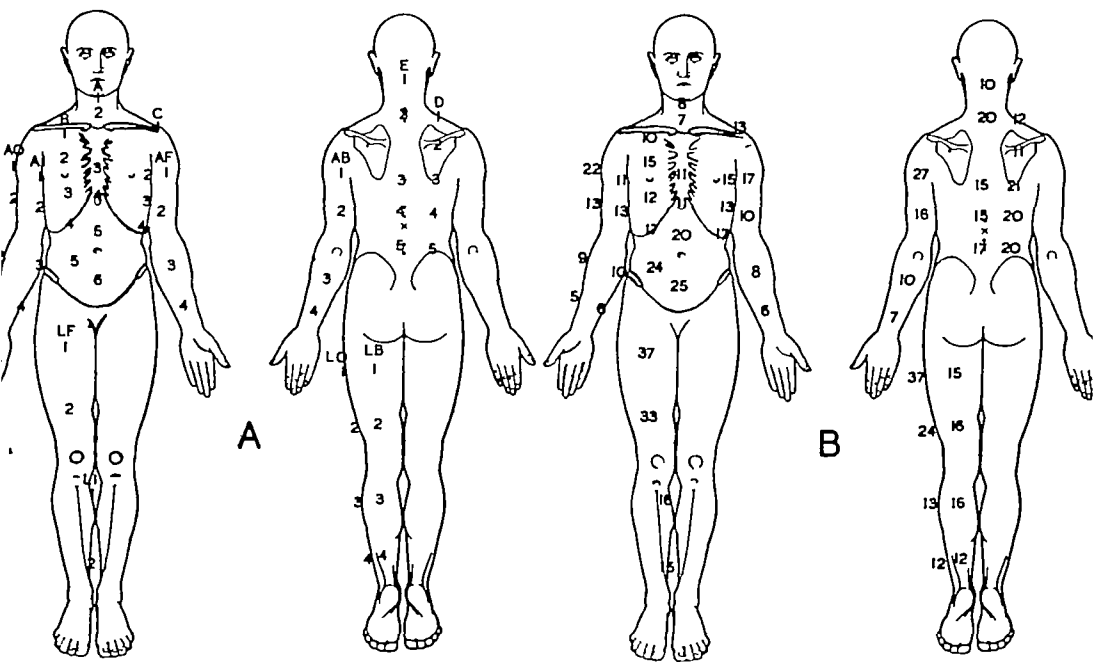
Fig 1   Sites of measurement and corresponding increases in fold thickness which occur as the weight increases from 100 to 180 lb   The reference number marks the site in the two left hand figures (A) and the increase in fold thickness in mm at the same site is given in the two right hand figures (B)

tissue is also limited   The sites must be accurately and quickly located with close correspondence between one subject and another and were therefore chosen in simple relation to easily identified bony landmarks Originally ninety-three points were selected and the repeatability of the measurements estimated   As a result of this trial experiment fifty-three sites were chosen as representative of the greater part of the body yet having reasonably good repeatability   Particular attention was paid to those areas of the body which are commonly said to be the sites of unusual fat distributions   The sites are illustrated in Fig 1

TABLE I

Site	Average fat fold thickness at 148 lb (mm.)	Increase in fold thickness from 110-180 lb (mm.)	Double skin thickness (mm.)	Weight at which corrected base line is intersected (lb.)
A 1	8 1	8	2	78
2	5 0	7	2	100
3	8 5	11	2	93
4	12 2	11	3	71
5	18 1	20	2	78
6	24 8	25	1	82
B 1	6 3	10	2 5	100
2	11 7	15	2 5	93
3	11 4	12	2	75
4	14 7	17	1 5	82
5	23 4	24	2	78
C 1	10 4	13	2	87
2	11 8	15	2	86
3	12 9	13	2	85
4	15 4	17	2	85
D 1	11 1	12	3	82
2	16 0	11	3	85
3	16 6	21	3	80
4	16 6	20	4	89
5	17 8	20	4	88
E 1	9 4	10	4	82
2	16 5	20	4	90
3	12 4	15	4	80
4	13 5	15	1	91
5	14 5	17	1	91
AF 1	14 2	17	1	88
2	8 4	10	1	85
3	7 6	8	3	85
4	6 3	6	2	75
AB 1	30 2	27	1	70
2	18 2	16	1 5	69
3	9 4	10	2 5	80
4	7 2	7	2	84
AO 1	23 4	22	2	71
2	15 1	13	1	65
3	7 9	9	1	80
4	6 3	5	1	61
AI 1	9 9	11	1	83
2	12 4	13	2	82
3	8 8	10	2	83
4	6 1	6	3	83
LF 1	38 3	37	3	75
2	33 9	33	2	75
LB 1	25 4	15	2	30
2	21 7	16	3	67
3	16 1	16	3	67
4	14 0	12	2	68
LO 1	38 6	37	2	72
2	27 4	24	2	66
3	16 5	13	3	64
4	14 4	12	3	63
LI 1	21 3	16	2	61
2	19 2	15	2	62

The tissue fold was measured with a pair of engineer's vernier calipers scaled in millimetres, modified for the purpose by building up the jaw face to 30 mm long and 8 mm wide   This considerably reduced the indentation of the fold by the jaws which were moved by a small friction wheel so that a roughly constant tension could be put upon the fold by an experienced operator   Fold thicknesses were recorded to the nearest millimetre   It must be emphasised that the values obtained by this method are not the real depth of subcutaneous tissue, but a direct proportional relationship holds between the fold thickness and the subcutaneous tissue thickness when these are measured on post mortem material

The subjects examined were all patients from the various In and Out Patient Departments of the Hospital who were not suffering from endocrine disorders or wasting diseases , many of the obese subjects were attending for weight reduction   Subjects were chosen at random except that later in the study they were selected on the basis of their corrected weight in an attempt to equalise the numbers in the various weight groups   The height in inches (without shoes) and the weight in pounds (unclothed) were recorded at the time of the fold measurements   So that subjects of different heights could be compared by weight, the actual weight of the subject was converted to a weight corresponding to a standard height of 64 inches by means of Table II   This converted weight was used in all calculations   The table was derived from height-weight-age data on women published by the Association of Life Insurance Directors and Actuarial Society of America (1)

TABLE II

Conversion table for reduction of weight to a standard

height of 64 inches

Height in inches	Add lb	Height in inches	Subtract lb
57	19	65	3
58	17	66	7
59	15	67	11
60	12	68	15
61	9	69	19
62	6	70	23
63	3	71	27

Results

The measurements were used to investigate two points firstly, the estimate of the total subcutaneous fat afforded by the sum of all readings on a subject and its relationship to the body weight, and secondly, the pattern of subcutaneous fat distribution shown by the readings at individual sites and the constancy of this pattern over a wide range of body weight

Total subcutaneous fat The first series of subjects studied consisted of 83 women whose menarche had occurred at least five years previously, who were having regular menstrual cycles, were not showing menopausal
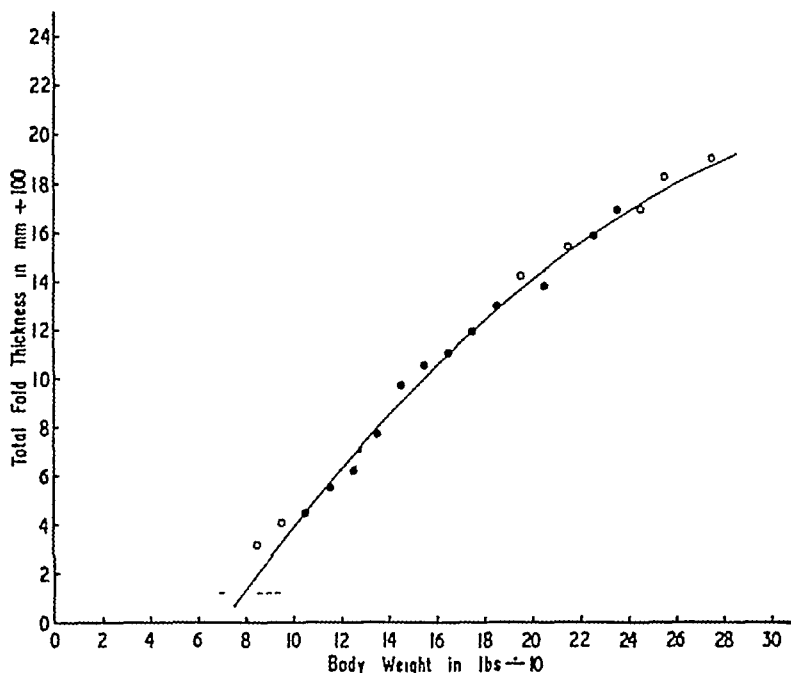
Fig 2 The relationship between total skin fold thickness and corrected body weight The black dots represent the mean values of 5 or more subjects, the open circles represent the mean values of 4 or less subjects Data at 255 and 275 lb are for single subjects only The regression line best fitting the data and the base line corrected for skin thickness are shown The data are the combined values for series N and P a total of 138 subjects

symptoms and did not admit to ever having been pregnant (Series N) The sum of the fold thicknesses at all sites was calculated for each subject and the subjects divided into groups according to their converted weight, each group covering a range of 10 lb The mean value of the total thicknesses was plotted against the mean value of the corrected weight in each weight group, these points fell close to a curve A second series of subjects, consisting of 55 women with the same menstrual history as the first group but who admitted to having been pregnant at some time, was treated in a

similar manner (Series P) , these points also fell close to the same curve
In Fig 2, which illustrates these findings, the two series of data have been
combined   The calculated curve which best fits the data is quadratic and
is given by $y = -0\ 022\ x^2 + 16\ 83\ x - 1072\ 6$, where x is the body weight
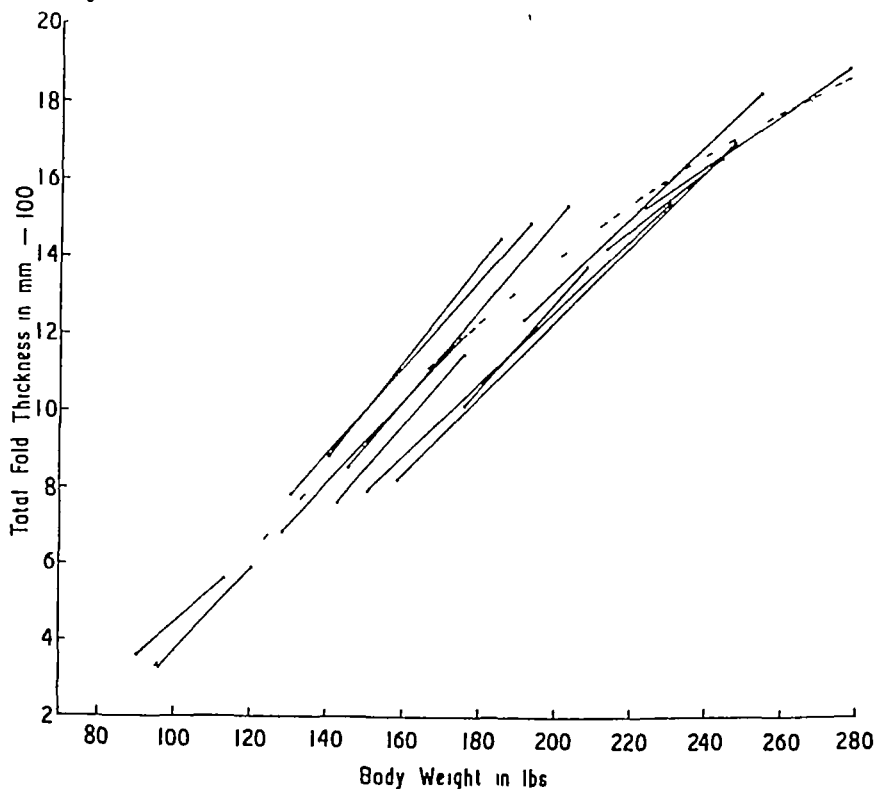in lb and y is the total fold thickness in mm

Fig 3   The effects of weight loss on the total subcutaneous tissue of the individual   The
broken line represents the regression line for the group   Values for each individual are
joined by continuous lines

Only patients who had not lost weight for some time prior to the
measurements being taken were included in these two series in order to
avoid any effects of weight loss on the tissue tension or the pattern
of subcutaneous tissue thickness   These average figures therefore indicate
the way in which the average total subcutaneous fat thickness for the group
varies with body weight   In order to see whether an individual conformed
to the group trend a series of 24 patients who were on a reducing diet and
had lost at least 2 stones were measured at intervals as they lost weight
The total fold thicknesses were plotted against corrected body weight as
in the previous groups and compared with the average line for these groups ,
some of these are illustrated in Fig 3   Two points were shown by these
data   the total fold thickness of an individual subject decreased with the

body weight in a similar manner to the group trend, this decrease was somewhat faster than the group trend but was not progressively so and was independent of the amount of weight lost   This decrease in the total fold thickness below the expected level has been found to occur in most patients who are losing weight rapidly and appears to be related to a change in the mechanical conditions involved in the method of fold measurement, such, for example, as a change in tissue tension or elasticity   The apparent decrease in fold thickness was roughly the same regardless of how much weight had been lost in excess of 10 to 15 lb
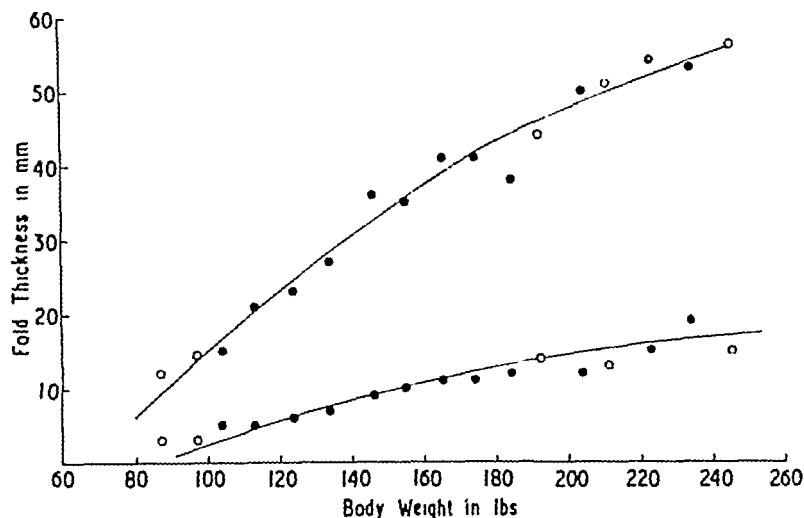
Fig 4   The relationship between fold thickness and body weight at two sites   The upper curve represents the back of the upper third of the upper arm (AB1), the lower curve the back of the lower third of the forearm (AB4)   The black dots represent the average of 5 or more subjects, the open circles 4 or less subjects   The data are the combined values for series N and P

The pattern of subcutaneous fat distribution   From the results so far obtained it was clear that there was a close and simple relationship between body weight and the total subcutaneous tissue   As the subcutaneous fat increased with obesity it might be deposited in one of two ways, either according to the existing pattern or first in some areas and later in others If the new fat was added according to the existing pattern then all the site values would increase proportionately, a doubling of the thickness at one site being accompanied by a doubling of the thickness at all other sites , if the fat was added irregularly then the pattern would differ at different body weights and these differences would be revealed by comparing graphs of tissue thickness against body weight for different sites of measurement The various sites of measurement were therefore treated independently, the thickness at a given site being plotted against corrected body weight for each subject in Series N   In each case the points were found to group

closely around a curve similar in shape to that for total fold thickness, the closeness of the grouping varying somewhat with different sites of measurement   The scatter was made up of both random errors and individual deviations from the average line and the data provided information about the group trend only   The average values for each 10 lb weight group were then plotted for series N and series P for each site   The points for the two series corresponded closely in the majority of cases and were therefore combined   For all sites of measurement the average values for the combined series were clearly grouped around curves, two of which are illustrated in Fig 4   The curves for different sites were similar in shape   Indeed, the curve of Fig 2, when suitably reduced in vertical scale, fitted the data for all sites with reasonable accuracy   This indicated that the average thickness of subcutaneous tissue at any one point on the body bore a simple and close relationship to the average body weight
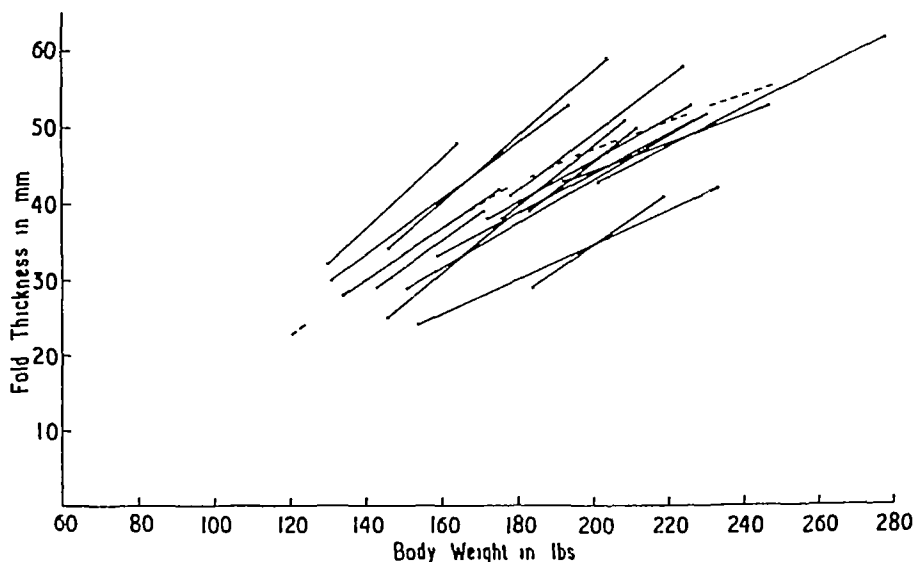
Fig 5   The effects of weight loss on the individual's fold thickness at the site represented in the upper curve of Fig 4   The broken line represents the regression line for the group   Values for each individual are joined by continuous lines

The data on the patients who had lost a considerable amount of weight on a reducing diet were plotted in the same way, the readings on each individual being joined by a line   It will be seen from some of these data (Fig 5) that although the individual's fold thickness at a given point may have differed considerably from the group average, the fold decreased in thickness with decrease in weight at a rate closely corresponding to that of the average for the group

In the grouped data the regression lines for each site all had the same general shape, but the slope of the lines varied from site to site   This result indicated that new fat was deposited on an existing pattern and that as the body weight changed the ratio of the new to the old thickness was similar for all sites on the body , in other words, if the average thickness for the group at one site was doubled, the thickness at any other site was doubled and the pattern was constant at all body weights in the range examined

The slopes of the group lines may be used to describe the pattern of distribution in two ways , either as the increase in thickness which occurs at each site for a given increase in body weight or as the thickness at each site at a particular body weight   The increase in thickness at the various sites which took place as the average weight increased from 110 to 180 lb is given in Table I and Fig 1

The second method of expressing the pattern of distribution requires some qualification   The thickness measured included two layers of skin and a correction was necessary for this   The double skin thickness was therefore measured at each site in eight female cadavers corresponding roughly to the age and weight distribution of the series N and M and the average double skin thickness at each site calculated   The average weight of all the subjects was used as the reference point for body weight and the height of the regression line at this weight (148 lb) determined at each site   This value minus the double skin thickness for the site represented the proportional thickness of subcutaneous tissue and is given in Table I with the value for the double skin thickness

One further observation was made on the data for the individual sites The regression line for each site was extrapolated to cut the base line of body weight after correcting for the double skin thickness   This value indicated the body weight at which there would theoretically be no subcutaneous fat at that particular site and is given in Table I   The regression line for the total fold thickness cuts the corrected base line at 80 lb (Fig 2)

DISCUSSION

The relationship between body weight and total subcutaneous tissue thickness   The results on the group data suggest, and the data on individuals strengthen the suggestion, that an adult female 64 inches tall would have virtually no subcutaneous fat at a body weight of about 80 lb   With increase in weight above this figure the amount of subcutaneous fat increases proportionately with the weight   This does not mean that the increase in weight is entirely due to the subcutaneous fat, but that whatever other tissues are involved must increase in proportion with the subcutaneous fat The tissues which might take part include adipose tissue in any part of the body and muscle , it is unlikely that the skeleton, nervous system and viscera

would contribute more than a small percentage of the increase and the same is probably true in the case of the connective tissues and extracellular fluid Muscle hypertrophy may occur but would again be a relatively small factor, although the muscle volume may increase as a result of fat deposited within or between the fibres The greatest contribution to the increasing weight would appear, therefore, to be from the adipose tissues of the body both in the deep and superficial tissues and it seems likely that all the adipose tissue which takes part in the increase does so at a rate proportional to the rise in weight *

The relationship between body weight and subcutaneous tissue thickness at different points on the body   The results suggest that the thickness of subcutaneous tissue at any one place on the body depends upon a constant local factor, which varies from place to place, and upon the body weight This might be explained by the hypothesis that the local factor is the number of cells in the subcutaneous tissue capable of storing fat If the storage of fat by adipose tissue cells is a simple biochemical process depending on the concentration of certain substances in the blood stream it is to be expected that each cell will take up an aliquot of the fat forming substance that is in excess provided that no mechanical factors interfere with the process In this way the subcutaneous tissue would increase in thickness in the same proportion over the whole body but the greater the number of cells in any one place the greater would be the absolute increase in thickness

The values for the average increase in each fold thickness as the weight increases from 100 to 180 lb show how the contours of the body are changed with increasing weight although the pattern of subcutaneous fat distribution remains the same There is an average increase in fold thickness of but 5 mm on the lower third of the radial side of the forearm whilst there is a simultaneous increase of 27 mm on the posterolateral aspect of the upper third of the arm The width of the shoulders will therefore increase more than that of the wrist At the same time the subcutaneous tissue fold over the angles of the ribs increases by 20 mm , that of the abdominal wall between umbilicus and pubis by 25 mm and that of the anterior and lateral aspects of the upper third of the thigh by 37 mm whilst at the lower third of the calf it is only 12 mm Fig 1 illustrates these and other increases and shows that the greatest fat deposition occurs around the shoulders, the upper part of the thigh, the lower abdomen, the base of the neck and the angles of the ribs In the limbs the deposition is progressively less from proximal to distal The tendency to deposit fat chiefly around the pectoral

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* Dr E C Pochin has used the preliminary observations at 93 sites distributed over the body surface to calculate a mean thickness for the subcutaneous tissues in 43 female subjects with varying amount of fat from the mean of the thicknesses actually observed A rough estimate of the total weight of subcutaneous fat was based on this value and a mean surface area as calculated from Du Bois's formula On this basis subcutaneous tissue, if assumed to have a specific gravity of 0 94, was found to represent about 70 per cent of the total excess body weight in any given subject

and pelvic girdles, " humping " of the back and relative " sparing " of the extremities is therefore a normal phenomenon as judged by the average pattern for the group and cannot be said to be a characteristic abnormal distribution of fat   There is no suggestion from the data obtained that a previous pregnancy makes any difference to the pattern of distribution , there may be a different pattern of distribution during pregnancy, but any difference which may occur must revert rapidly to the normal afterwards

The data obtained by extrapolating the regression line to cut the weight axis corrected for skin thickness give confirmatory evidence of the reliability of the measurements   The values for adjacent points are surprisingly close, for example the values for the back are 89, 89, 88, 90, 89, 91, 91, for the flank 87, 86, 85, 85, for the inside of the arm 83, 82, 83, 83 lb   The difference in the values from area to area is probably a measure of the amount of non-fatty tissue enclosed in the subcutaneous tissue fold, the smaller the weight at which there would theoretically be no fat the greater the amount of non-fatty tissue   The exceptionally low value for site LB1 is the result of incorporating part of the rounded edge of semitendinosus in the fold

The results give information about the average pattern of distribution and deposition of subcutaneous fat in a group of 138 women but these conclusions cannot necessarily be applied to the individual   It is obvious that many normal subjects show variations from the general pattern described but the limited data on subjects losing 28 lb or more in weight suggest that whatever the individual s basic pattern of distribution, this persists over a wide weight range   There are many variations or additions to the pattern described which occur relatively commonly and must be considered normal since they are not associated with any particular condit on and exist in the fat and the skin   They include a pad over the sacrum , a pad over and below the inferior angle of the scapula , a pad filling in the groove between the biceps and triceps on the lateral aspect of the arm , a pad over the medial aspect of the upper third of the adductor muscles , and a pad over and above the medial side of the knee, limited below by a crease which becomes continuous with the popliteal crease   There is frequently a bulge in the popliteal fossa above the joint line which is separated from the medial knee pad by a crease   Around the ankle there is sometimes an extension of the thick fat layer over the calf down on to either side of the Achilles tendon giving a ' thick ankle " appearance , another pad occurs below and behind the medial malleolus overlying the flexor retinaculum and another below and in front of the lateral malleolus overlying the lateral parts of the superior and inferior extensor retinacula   These local increases in thickness may be found in many slim people and are not necessarily associated with obesity   In the obese they are more obvious, but with decrease in body weight they have been observed to become less conspicuous as the fat thickness decreases elsewhere

SUMMARY

A method of estimating the thickness of subcutaneous tissue fat and the results of an investigation into the normal adult female pattern of subcutaneous fat distribution are described   A close relationship between subcutaneous tissue thickness and body weight was observed at 53 sites on the body   The thickness of subcutaneous tissue was found to vary from place to place according to a pattern which remained constant over a weight range of 110 to 180 lb and probably over a much wider range over which the data were meagre   This normal pattern included a relatively greater deposition of fat around the shoulders, base of neck, back, abdomen and thighs than elsewhere with a " sparing " of the extremities   Some common variations of the normal distribution are described

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ON THE EFFECT OF ARTERIAL OCCLUSION AND VENOUS CONGESTION UPON LIMB PAIN

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Introduction

THE causes of pain in disease and their relationship to disorders of the circulation are still not clearly understood   Careful studies have been made in special syndromes such as headache (2, 4, 13, 15, 16), intermittent claudication (8), disorders of the peripheral nerves (3, 5, 6, 7, 11, 12, 18, 19), peptic ulceration (1, 14) and angina of effort (17)   From these studies it would appear that pain in disease may be caused by at least three different mechanisms   First, there is what may be called the chemical mechanism   In this, pain results from the accumulation of substances such as metabolites in contracting muscle or damaged skin, or the concentration of acid in the stomach   Secondly, there is the vascular mechanism so prominent in headache where pain may result from an abnormal amplitude of pulsation in the vascular bed   Lastly, there are the various painful states, including causalgia, which may follow injuries and other disturbances of the peripheral nerves and which are frequently associated with morphological changes in the nerves themselves   In spite of these studies the painful conditions in the limbs resulting from trauma, infection and rheumatic disease have been strangely neglected   We have, therefore, investigated some of these common painful lesions and have observed the effect of short periods of arterial occlusion and venous congestion upon the severity of the pain

A painful condition was defined as one in which movement or palpation of the part caused excessive pain   In most instances the subject had also experienced spontaneous pain during the preceding twenty-four hours or was suffering slight pain at the time of observation

Method

Since it has been shown that temperature changes may sometimes have a profound effect upon both deep and cutaneous pain (7, 9, 10, 19), we have kept the temperature of the affected part constant during our

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observations   If the part affected was the hand or foot its tempeiature was controlled by immersion in a water bath , but when the knee or elbow region was affected the limb was merely kept under the bed clothes or wrapped in gamgee   By these simple procedures the skin temperature of the part was kept constant to within 1 °C during the period of investigation All tests were carried out within the range of 30° to 35°C unless otherwise stated

A pneumatic cuff was applied to the limb proximal to the lesion Arterial occlusion or venous congestion were produced by inflating the cuff to a pressure of 200 or 80 millimetres of mercury   The pressures were applied suddenly from reservoirs and each pressure was maintained for 10 minutes and then released rapidly   The temperatures were allowed to stabilise before each observation and, except in certain experiments, the blood was permitted to circulate freely for at least 5 minutes between the application of each pressure
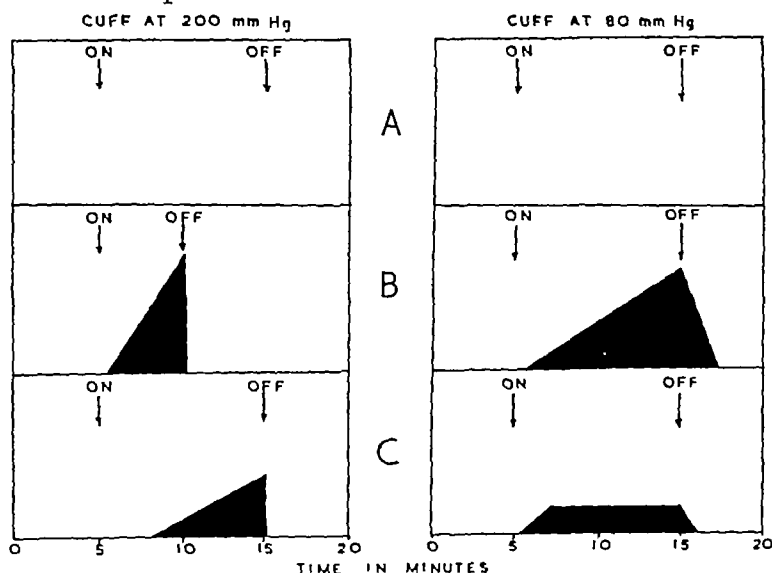

I ig 1   Illustrates the 3 types of response to short periods of arterial occlusion and venous congestion   A.—No effect   B —Rapid increase of pain   C —Slight or modified increase of pain   The black areas represent spontaneous pain in this and subsequent figures

A careful note was made of any change in spontaneous pain or tenderness that occurred during these procedures   Pain was graded as slight, moderate and severe, but tenderness proved difficult to assess satisfactorily because of the variety of lesions tested

These tests were done on patients suffering from infections of the hand and other suppurative lesions of the limbs, painful nerve injuries, operation wounds, minor fractures and sprains, rheumatic and other arthritic diseases, and many miscellaneous painful states

Types of response

The effects of arterial occlusion were studied in 151 patients, and in 138 of these the effects of venous congestion were also observed

Three main patterns of response were noted and these are illustrated diagramatically in Fig 1   In the majority of patients there was no response, the painful condition remaining unchanged throughout the periods of arterial occlusion and venous congestion   In others arterial occlusion produced a rapid and dramatic increase of pain which was often accompanied by generalised pallor sweating and such distress that the circulation had to be restored after only a few minutes of occlusion   After release this severe pain vanished when the flush of returning blood reached the affected part   In many patients, however, pain was only slightly increased by arterial occlusion and this was classified as a slight or modified response

In some patients venous congestion produced some increase of pain but in only 3 was this increase of sufficient severity to require release of the cuff pressure before the full 10 minutes had elapsed   Furthermore, with venous congestion there was usually an early increase of pain which then remained at a plateau for the duration of congestion   After release the pain subsided slowly, sometimes taking as long as a minute or two to disappear   The response to congestion thus differed from the response to arterial occlusion where a continuous increase of pain was followed by immediate relief on restoring the circulation   Although there was some correlation between the responses to arterial occlusion and venous congestion, in a number of patients who experienced a dramatic increase of pain during occlusion, venous congestion had no effect whatsoever, while in 3 of these it even caused some relief of painful throbbing   There were also 4 subjects in whom venous congestion alone increased pain, arterial occlusion being without effect   But most painful lesions that were affected by the tests responded to both arterial occlusion and venous congestion

In repeated observations each subject gave the same type of response unless there had been some obvious evolution of the lesion between the observations

Relation to pathological lesion

These three types of response were commonly associated with different types of pathological lesion, and a classification of our cases is shown in the table

The most pronounced increase of pain during arterial occlusion was observed in pulp space infections of the finger, and infection of the fascial compartments of the hand   These lesions account for 19 of the patients classified as suffering from closed deep suppuration   The remaining patients in this group comprised two with active osteomyelitis, one with suppurative

Type of lesion	Pain from occlusion			Pain from congestion		
	Severe	Slight	None	Severe	Slight	None
Closed deep suppuration	18	7		1	8	7*
Operation wounds	6	4		1	8	1
Minor fractures and sprains	1	9	10	1	8	10
Superficial suppuration		9	10		5	11
Rheumatoid arthritis		11	14		11	14
Heberden's nodes		3	3		3	3
Osteoarthritis		1	4		1	4
Tuberculous arthritis			4			4
Ankylosing spondylitis			3			3
Nerve injury Type I			10			10
Nerve injury Type II			10			10
Glomus tumour			5			5
Miscellaneous	2		7		1	8
Totals	27	44	80	3	45	90

The Table shows the number of cases of each type of pathological lesion in which arterial occlusion or venous congestion produced either no change, a slight increase of pain or a severe increase of pain, necessitating release of the cuff before the elapse of 10 minutes

In 13 of these cases venous congestion was omitted

arthritis, one with disseminated lupus who had sterile symmetrical abscesses in the hands, and 2 patients with Reiter's disease who had sterile purulent effusions in their joints

Similar dramatic responses were observed in patients with operation wounds of the hands where there was considerable post-operative swelling and tenderness, but in those with trivial incisions and little post-operative swelling the response was very slight The increase of pain was most pronounced on the day after operation and gradually became less marked on each subsequent day, to become rather suddenly negative on the 2nd to 7th day, depending upon the severity of the post-operative tissue reaction In the group of minor fractures and sprains the response varied considerably In those with noticeable local warmth and swelling there was usually some response, while negative responses were found immediately after injury,

* In 3 of these painful throbbing was relieved by venous congestion

and again after the lapse of days or weeks when no local warmth or swelling was present, but serial studies of more severe injuries will have to be made before the responses in this type of trauma can be properly defined

The group of superficial suppuration contained a miscellaneous collection of subcuticular blisters, incised and freely draining infections of the hands and three patients with mild cellulitis

Most of the 25 patients with rheumatoid arthritis had acutely inflamed joints which were hot and gave severe pain on pressure and movement It was therefore surprising to find that none of these experienced more than a slight increase of pain during arterial occlusion or venous congestion Three patients with acute Heberden's nodes that resembled infected fingers gave positive responses, but in patients with the common chronic bony nodes no response was observed   Five osteoarthritic knees were tested Only one of these gave a positive response and this patient was suffering from an acute episode with a hot, swollen joint following a recent injury No response was observed in 4 patients with active tuberculous arthritis and in 3 patients with ankylosing spondylitis in whom the knee was affected

In 20 patients suffering from persistent painful states following nerve injuries and in 5 patients with painful glomus tumours no response was observed   Under the heading of Type I nerve injury we have included those patients in whom an area of skin was so hypersensitive that even light contact with a hair produced a complaint and withdrawal of the part   In this group temperature changes within the range of 45° to 15°C caused no abnormal pain   Under Type II nerve injury we have placed those patients in whom the pain spread widely and was felt deep in the limb and in whom excessive pain resulted from cooling the affected part, within the range 30° to 15°C   Most of these cases have been described in detail elsewhere (5, 7)

Among the miscellaneous group there were 3 patients with arterial disease who were suffering from the rest pain of impending gangrene   There was also one patient who was considered to be suffering from Sudeck's atrophy, one patient with chronic inactive osteomyelitis who had tender scars, and two patients with painful joints resulting from pulmonary osteoarthropathy   In none of these patients was any response observed

The two markedly positive responses in this group occurred in patients with malignant disease   One of these had a fibrosarcoma of the palm, whilst the other had a malignant synovioma affecting the elbow   Both these tumours were unusually painful growths and they were probably not representative of sarcomata as a whole

A glance at the table shows that in 25 patients with deep closed suppurations there was a considerable and usually dramatic increase of pain during arterial occlusion, whilst in 20 patients with peripheral nerve

injuries and in 5 patients with glomus tumours neither arterial occlusion nor venous congestion had any effect whatsoever   As in both groups tenderness was pronounced, it was unlikely that this difference in response to occlusion was simply due to a differing degree of the same painful state

As the increase of pain which occurred during arterial occlusion in patients with closed suppuration was a most striking phenomenon it was decided to investigate this more fully in those suffering from pulp space infections of the finger

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DETAILED STUDIES

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For these studies we chose patients who were not suffering from spontaneous pain but in whom even a few minutes of arterial occlusion produced severe pain, and a note was made of the time after occlusion when pain was first felt, and also when it became unbearably severe

On 4 subjects the test was carried out in the usual way but the circulation was then re-occluded after free intervals varying from 10 seconds to 5 minutes   When the free interval was longer than 30 seconds the pain came on after the same time and increased in the same way as in the original occlusion, but with shorter free periods the pain tended to come on earlier and to increase more rapidly in the second occlusion

In 2 patients serial observations were made daily during penicillin therapy   As the inflammatory process in the finger subsided there was progressive delay before the onset of pain during occlusion and the rate of increase became slower   In 3 patients, similar serial observations were made before and after incision of the finger   Immediately after the incision the pain came on later during occlusion and increased more slowly than previously, and during the following two or three days the response ceased altogether

In the two patients with Reiter's disease, in whom a positive response was noted, this became negative 24 hours after aspiration of a purulent effusion from the affected joint

During all previous tests the affected finger had been kept in a bath of water at 35°C, but the tests were now repeated with the finger immersed in water at different temperatures   First the finger was placed in water at 40°C and after allowing 5 minutes for the finger to warm through, the arteries of the arm were occluded in the usual way, until the pain became unbearable   After 5 minutes rest the procedure was repeated with the finger in water at 30°C and at 20°C and occasionally at intermediate temperatures   The different temperatures were also used in the reverse order   Fig 2 illustrates a typical result   It will be seen that during occlusion the pain comes on earlier and increases more rapidly at the higher temperatures than at the lower temperatures   Comparable results were obtained in all of the 7 patients with infection of the finger tip, which were

studied in this way *   It is well known that a burn is abnormally sensitive to warmth, and Lewis and Hess (9) have shown that if skin be damaged by any means such as burning, scratching, freezing or exposure to ultra violet light, it becomes abnormally sensitive to warmth and may give rise to spontaneous pain at temperatures as low as 30°C   These workers came to the conclusion that pain and abnormal sensitivity resulted from the action upon the pain nerves of substances passing out from damaged cells
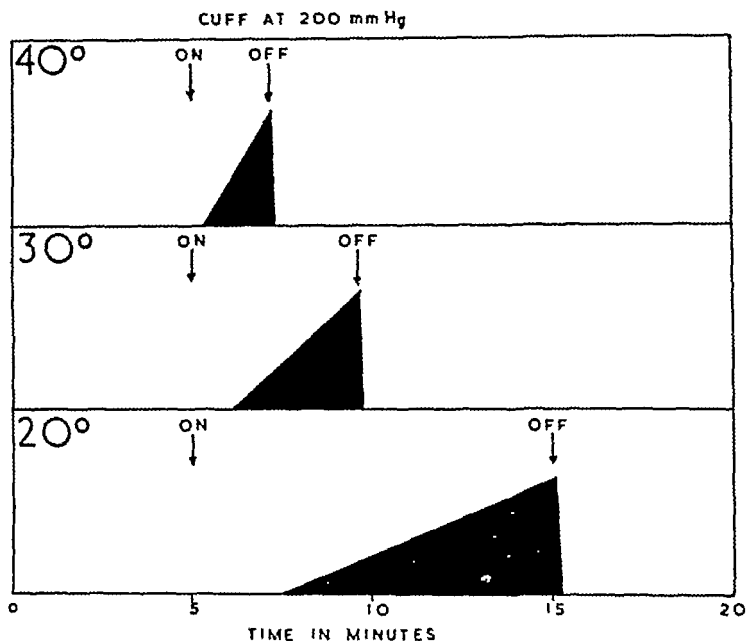

Fig 2   Shows the time of onset and rate of increase of pain during occlusion, when the infected finger is held in a water bath at different temperatures

The following test was, therefore, carried out on 8 patients   (Fig 3)   The affected finger was placed in water at 40°C and after the usual 5 minutes the arteries of the arm were occluded   As soon as spontaneous pain was felt the finger was transferred to water at 30°C   This produced rapid relief, but after a minute or two pain returned   The finger was again transferred to water at 20°C with a further temporary relief of pain, but once pain was felt at 20°C no further lowering of temperature gave any relief and the pain continued to increase until the circulation was restored by releasing the cuff   If, during this experiment, the finger was transferred from a lower to a higher temperature there was an immediate return of pain

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* In these finger tip lesions pain is mostly cutaneous in type but when pain is largely deep as in suppuration in the fascial spaces of the palm or in arthritis, the pain induced by arterial occlusion tends to come on earlier and to increase more rapidly at 20°C

It is difficult to see how cooling the finger can disperse some accumulating metabolite, but if during the period of occlusion there is a steady accumulation of a metabolite which increases the sensitivity of the finger to warmth, pain will be felt at lower temperatures as the occlusion period lengthens   During occlusion the finger also gives rise to increasingly severe pain on squeezing, and it seems likely that during occlusion there is a progressive increase of sensitivity to a variety of stimuli
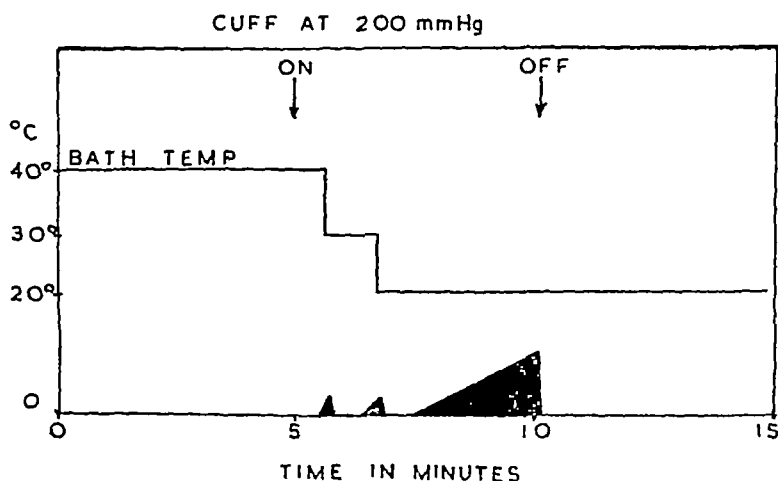
Fig 3   Shows the interference with the increase of pain during occlusion which results from transferring the infected finger from a warm to a cool water bath

The infected finger thus behaves like the damaged skin studied by Lewis and Hess (9), and a similar mechanism may well be operative in the many painful conditions in which there is an increase of pain during arterial occlusion   Furthermore, in deep suppuration a collection of pus may act as a reservoir for pain producing substances and the relief of pain which frequently follows incision and drainage may be due more to the emptying of this reservoir than to the relief of tension, although this undoubtedly plays some part

Discussion

These observations suggest that painful conditions of the extremities can be classified into at least two main groups   In the first group the painful state is extremely labile and is profoundly affected by short periods of arterial occlusion , and in this group venous congestion may also cause a considerable increase of pain   In the second group the painful state is more stable and is unaffected by short periods of arterial occlusion or venous congestion

The labile state is commonly associated with an inflammatory tissue reaction but, although the stable state may be found in a variety of conditions, it is characteristically associated with peripheral nerve injuries

In the labile painful states the damaged tissues are probably producing substances which stimulate the pain nerve endings directly and also render them abnormally sensitive to other stimuli such as pressure, tissue tension and changes of temperature For the disposal of these substances an ample local blood flow is essential Thus a delicate balance is maintained between the blood flow through the affected part and the accumulation of these substances in the tissues

The problem is, however, extremely complex because the presence or absence of pain at any given moment may also be influenced by the temperature of the part, and the presence or absence of venous congestion, which alters tissue tension as well as local blood flow Thus at least four different, but interacting factors must be considered when discussing the cause of pain in these labile states

Pain in the head is frequently caused by an abnormal amplitude of arterial pulsation (2, 4, 15) When this mechanism is operative, arterial occlusion and venous congestion both tend to relieve pain by reducing the amplitude of pulsation in the vascular bed With the exception of 3 patients, in whom venous congestion relieved throbbing, no such relief has been observed in painful conditions of the extremities , and it therefore seems unlikely that abnormal arterial pulsation is an important cause of pain in the limbs, at least in the conditions studied

SUMMARY

1   Short periods of arterial occlusion and venous congestion have been applied to limbs in which a painful state had resulted from a variety of pathological lesions such as trauma infection and rheumatic disease

2   In many cases arterial occlusion produced a dramatic increase of pain In others only a slight increase of pain was observed while in a large group neither venous congestion nor arterial occlusion had any effect

3   On the basis of these tests painful states have been classified into labile and stable groups The labile state was usually associated with an inflammatory tissue reaction while, although the stable state was found in a variety of conditions, it was characteristically associated with changes in the peripheral nerves

4   The effect of temperature on a labile painful state has also been studied and some of the factors involved in the production of pain have been discussed

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ADDISONIAN PERNICIOUS ANÆMIA
CONFIRMATORY EVIDENCE OF A FACTOR INHIBITING ERYTHROPOIESIS

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THE work of Rusznyak, Lowinger, and Lajtha (5) has provided strong evidence that a factor which inhibits erythropoiesis is present in the serum of patients suffering from pernicious anæmia in relapse   They found that megaloblasts aspirated from the marrow of such patients matured rapidly when cultured in normal serum but not in serum from patients with pernicious anæmia   Moreover, the greater the concentration of pernicious anæmia serum in the culture, the greater the inhibition of maturation, while similar increases in the concentration of normal serum had no such effect   While non-maturation in pernicious anæmia serum could be explained by lack of a maturation factor, the finding of a greater percentage of immature cells with an increase in the concentration of serum suggested the presence of an inhibiting agent   Hays (2) found that normal human or rat serum produced a reticulocyte increase in bone marrow cultures but that serum from one patient suffering from pernicious anæmia in relapse did not   Norris and Majnarich (3) described an inhibition of cell proliferation by pernicious anæmia serum in bone marrow culture   They have shown that a similar effect is produced by serum from cases of leukæmia and neoplastic diseases   Osgood and Brownlee (4) also noticed poor growth of cells in pernicious anæmia serum   Since evidence of an inhibitor agent in the serum would necessitate some revision of current theories regarding the nature of pernicious anæmia a further series of experiments was planned, the results of which are recorded here

Methods

Marrow cells aspirated from patients suffering from pernicious anæmia in relapse were cultured in varying proportions of normal and pernicious anæmia plasma   The influence of the medium upon cell maturation was assessed by differential erythroblast counts on smears prepared before and after 18 to 20 hours culture at 37 5°C , the cells being graded according to nuclear maturity

The method of bone marrow culture used was essentially that devised by Osgood and Brownlee (4)   Aspirated marrow cells were cultured in a medium consisting of plasma or serum diluted with physiological saline (Gey's solution) contained in serum bottles of 20–25 cc capacity   In a few experiments a modification was employed permitting multiple cultures from a small volume of marrow (6)   When plasma was used heparin was employed as an anticoagulant in a concentration of about 1/20,000 , at this dilution it does not inhibit cell growth

Erythroblasts were classified by a method similar to that employed by Davidson, Davis and Innes (1) in their observations on the changes occurring in pernicious anæmia marrow after liver therapy   This classification is briefly as follows —

Type I   These are large basophilic cells measuring 18μ or more in diameter   The nucleus is pale and consists of fine reticulated chromatin , nucleoli may be present

Type II   These are rather smaller cells , the cytoplasm may be basophilic or polychromatic   The nucleus is more deeply staining than Type I and the chromatin net is coarser , no nucleoli are present

Type III   These are smaller still, the cytoplasm may be basophilic, polychromatic, or eosinophilic   Nuclear chromatin is much more densely clumped

Type IV   The smallest and most densely staining cells seen and corresponding to the usually accepted description of a late normoblast

Experience showed that a count of 400 cells was sufficient for an accurate assessment of the maturity of a culture , 100 cells were counted from each of 4 slides   The results as presented here are simplified by combining the percentages of Types I and II erythroblasts   It was found that the percentage of these two types gave a satisfactory index of the maturity of a culture   The standard error for each group of results is given , observations differing by more than twice this figure are considered significant

Occasional cultures became infected, all such experiments were discarded Death of a culture also occasionally occurred , it was always obvious from the loss of cellular outline, vacuolation of the cytoplasm, and loss of nuclear pattern—resembling the appearance of marrow aspirated post mortem

All the patients were later used for clinical trials of vitamin B_{12} , the hæmatological criteria and dietary control are described elsewhere (7)

1   *Culture in normal plasma*

The effect of varying the concentration of normal plasma on the maturation of marrow cells was first determined   The results given in Table I demonstrate that there is no significant difference in the degree of maturation in cultures containing between 10 and 100% of plasma

TABLE I

Experiment	Initial count	Percentage of Types I and II erythroblasts						Standard error
		After culture in normal plasma 10–100%						
		10%	20–30%	40%	60–65%	80%	100%	
I	52 5	—	33 0	39 0	—	34 0	33 75	3 2
II	52 0	—	28 75	—	24 75	—	—	2 6
III	61 0	—	48 75	46 5	44 25	—	—	3 2
IV	48 0	13 25	12 75	—	15 5	14 0	—	1 0
V	43 5	21 0	18 25	—	22 75	21 5	—	2 1

2   *Culture in pernicious anæmia plasma*

In this series of experiments, cultures were carried out in varying percentages of pernicious anæmia plasma   It was found that there was always a higher percentage of immature cells in the culture containing a high concentration of plasma than in those with a low concentration   A control culture was usually made in normal plasma and always showed greater maturation than in an equal strength of pernicious anæmia plasma   These findings suggested that maturation was inhibited in the pernicious anæmia plasma and that the effects could not be explained by a simple absence of a maturation factor   This inhibitory effect varied with different specimens of plasma , presumably this is explained by a varying concentration of the inhibiting agent

TABLE II

Experiment	Initial count	Percentage of Types I and II erythroblasts							Standard error
		After culture							
		Normal plasma	Pernicious anæmia plasma						
			10%	20%	40%	50%	60%	80%	
I	48 0	14 50	—	18 5	—	32 0	—	41 0	3 2
II	57 2	—	—	32 6	—	32 4	—	44 8	3-0
III	43 75	19 0	25 5	—	30 5	—	—	41 25	4 9
IV	66-0	23 75	31-0	—	37 25	—	—	49 0	1 8
V	33 0	4 25	—	6 75	—	8 75	11 5	—	1 6

3   *Effects of adding pernicious anæmia plasma to normal plasma*

The results of the above experiments having suggested the presence of an inhibitor in pernicious anæmia plasma, confirmation was sought by adding it in varying proportions to normal plasma   Were an inhibitor present it should, if added in sufficient amount, annul the maturing effect of normal plasma   The results summarised in Table III demonstrate that

TABLE III

Experiment	Initial count	Percentage of Types I and II erythroblasts								Standard error
		After culture								
		N P 20%	N P 40%	P A P 20%	P A P 40%	P A P 20% N P 20%	P A P 10% N P 20%	P A P 5% N P 35%	P A P 80%	
I	51 4	—	29 6	—	45 4	47 8	—	30 2	56 0	2 6
II	69 0	—	41 75	—	67 0	58 25	—	56 0	—	2 6
III	58 0	—	12 25	—	34 5	30 25	—	24 0	—	3 4
IV	46 0	15 25	—	25 0	—	30 0	13 75	10 5	—	3 2
V	48 0	6 25	7 25	21 25	27 50	20 0	—	—	—	0 8

N P —Normal plasma
P.A.P —Pernicious anæmia plasma

such inhibition did occur   In experiment 1 culture in 40% normal plasma produced a reduction of immature cells from 51 4 to 29 6%, whereas culture in 40% pernicious anæmia plasma produced a barely significant maturation to 45 4%   Culture in a mixture of 20% normal and 20% pernicious anæmia plasma resulted in no greater maturation than in pernicious anæmia plasma alone   Since 20% and 40% normal plasma have the same maturing effect this absence of maturation in mixed plasma must be due to an inhibitory effect from the added pernicious anæmia plasma   In a further culture 5% of pernicious anæmia plasma was added to 35% of normal plasma , maturation was as complete as in normal plasma alone   Finally, after culture in 80% pernicious anæmia plasma there were as many immature cells as in the initial count and a greater percentage than in 40% pernicious anæmia plasma

The other four experiments show similar results   Experiment 2, and to a lesser extent experiment 3, demonstrate that an inhibiting effect can be produced by even 5% of pernicious anæmia plasma   In experiments 1 and 4 however, this concentration had no effect so that plasmas from different patients probably contain varying amounts of inhibitor   The anomalous count in experiment 4 after culture in 40% pernicious anæmia plasma is probably due to experimental error

DISCUSSION

It has always been assumed that the very remarkable and characteristic abnormality of hæmopoiesis seen in pernicious anæmia was caused by the absence of an antipernicious anæmia principle directly necessary for the normal maturation of marrow cells The observations reported above suggest the presence in pernicious anæmia plasma of an agent capable of antagonising the maturing effect of normal plasma in culture It seems reasonable to suppose that there is a similar inhibiting effect in the body and that the mechanism of the hæmopoietic disorder in pernicious anæmia is more complex than has been thought It is possible that the inhibition is produced by a competition mechanism, blocking an enzyme system necessary for the synthesis of some nuclear constituent of marrow cells Further speculation does not appear justified until evidence has been obtained of the nature of the inhibitory agent

SUMMARY

Evidence is produced to confirm the presence in pernicious anæmia plasma of an agent which inhibits *in vitro* the maturation of primitive marrow cells obtained from patients suffering from pernicious anæmia in relapse This agent can annul the maturing effect of normal plasma It is suggested that a similar inhibiting effect occurs in the body and that the mechanism of the hæmopoietic disorder in pernicious anæmia is more complex than a simple deficiency of a substance necessary for the normal maturation of red cell precursors

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AN INHIBITORY FACTOR IN PERNICIOUS ANÆMIA SERUM

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PRELIMINARY work with bone marrow culture suggested the presence of a factor in the serum of patients suffering from pernicious anæmia which inhibited the conversion of megaloblasts to normoblasts (7, 8)   The present report is concerned with confirming these findings and investigating the nature of this factor and the relationship between it and hæmopoietic principles such as folic acid and vitamin B_{12}

Method

Cell suspensions from 31 human sternal marrows were cultured *in vitro* at 37°C using a modified Osgood technique (6)   In the earlier experiments the fluid culture medium consisted of 35% fresh human serum and 65% Gey solution (2)   Later the proportion of serum to Gey solution was varied and in some experiments the Gey solution was replaced by Ringer solution The maturation of the cells was followed by means of total counts and differential counts on stained preparations in 6, 12, 24, 48, 72 and 96 hour cultures

The percentage differences given in the tables (Tables I–VIII) were confirmed by calculated absolute counts which show that they represent real, and not merely relative, changes in the numbers of normoblasts and megaloblasts   Such changes may be due to a conversion of one cell type into the other, or to a dying off, or proliferation of either   Available evidence strongly suggests that it is the conversion of one type to the other that is taking place, but it is too complex to be discussed at length here, and a full account, together with a description of the culture technique and the maturation of 24 normal marrow samples will be published elsewhere

The term ' ripening " will be used to signify the transformation of megaloblasts to normoblasts and the term " maturation " for the development of later cell forms from younger forms in both normoblastic and megaloblastic series of cells

Experiments and Results

I —Culture of megaloblastic marrow in diluted normal and pernicious anæmia serum

Marrow samples from 10 patients with untreated pernicious anæmia were cultured both in 35% normal serum and in 35% pernicious anæmia serum   The megaloblasts ripened in both media although usually to a lesser extent in the pernicious anæmia serum (Table I)

TABLE I

Ripening of megaloblasts in normal and in pernicious anæmia serum
(35% *serum and* 65% *Gey solution*)

| No of exp | Megaloblasts/100 nucleated red cells | | |
| | Initial count | 48 hr cultures in | |
		Normal serum	P A serum
B4	23 5	3 5	0 5
B5	41 5	9 0	16 5
B6a	79 0	12 0	36 0
B6b	79 0	0 0	16·0
B19	29 0	0 5	2 5
B11	14 0	1 0	0 0
B13	32 5	4 5	9 5
B16	35 0	7 0	7 5
N3	31 0	19 0	20 0
N65	48 0	32 0	30 0

This behaviour might be explained by the supposition that there is only a partial lack of hæmopoietic factor in pernicious anæmia serum   This assumption does not explain why the megaloblasts ripen *in vitro* while the patient remains still in relapse   Alternatively, it seemed possible that there is a local inhibition *in vivo* which is not present *in vitro,* or as seems more likely, there is an inhibitor present in the serum the effect of which is reduced by the dilution used in the cultures   Experiments were designed to test this last supposition

II —The effect of increasing the concentration of serum in the medium

In an initial experiment the concentration of serum was increased by reducing the percentage of Gey solution in the culture medium to 35% (concentrated serum)

The megaloblasts which represented 37 5% of the nucleated red cells in the initial count, ripened in 48 hr culture in concentrated normal serum to 16 5%, in contrast they did not ripen in concentrated pernicious anæmia serum, the 48 hr culture showing still 36 5%

In three further experiments series of serum dilutions were prepared as culture media, and the results indicated that increasing the concentration of pernicious anæmia serum decreased the ripening of megaloblasts

To obtain further confirmation of the inhibitory effect, experiments with normal and pernicious anæmia serum in diluted and concentrated form were carried out   An increase in the concentration of normal serum in the medium increased the ripening of megaloblasts while an increase in the concentration of pernicious anæmia serum again decreased the ripening of megaloblasts (Table II and Fig 1)

TABLE II

Serum dilution experiments

(Diluted serum   35% serum and 65% Gey solution
Concentrated serum   65% serum and 35% Gey solution)

	Megaloblasts/100 nucleated red cells				
		Cultures in			
No of exp	Initial count	Normal serum		P A serum	
		Diluted	Concentrated	Diluted	Concentrated
B11	14·0	1 0	0 0	0 0	2 0
B13	32 5	4 5	2 0	9 5	16 5*
B16	35 0	7 0	5 0	7 5	13 0
B27	23 5	1 0	0 5	1 5	4 0
N3	31 0	19 0	11 0	20 0	28·0
N76	35 0	13 0	5 0	7 0	24 0
B1 ɔ	65 0	17 0	10 0		
B22	76 0			8 0	27·0
B23	2 5			0 0	3 0
B24a	70 0			3 5	21 0
B24b	70 0			7 5	15 5
N77	18 4			5·0	18·0
N79	14·0			30 5	46·0†

* Counted by two independent observers
† Counted by three independent observers

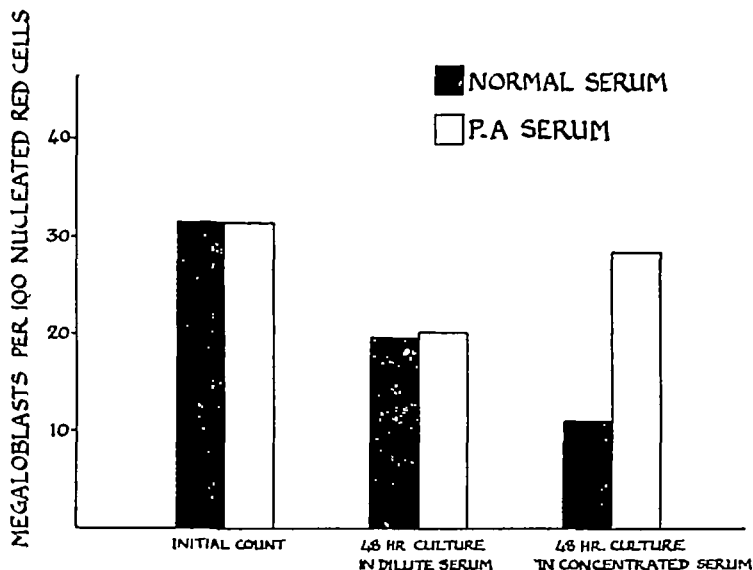## THE EFFECT OF VARYING THE CONCENTRATION OF NORMAL AND PERNICIOUS ANAEMIA SERUM IN THE CULTURE MEDIUM

FIG 1

It was concluded from these observations that there is a positive inhibitory factor present in the serum of patients with untreated pernicious anæmia the effect of which can be diluted out

III —*The effect of pernicious anæmia serum on normal bone marrow*

When normal bone marrow was cultured in concentrated pernicious anæmia serum no significant change in the maturation rate of normoblasts could be detected but a varying percentage of megaloblasts developed in 72 hr cultures (Table III and microphotographs)   This effect was not obtained with diluted pernicious anæmia serum, giving further evidence for the presence of an inhibitory factor in such serum   The highest proportion of megaloblasts developed in marrow samples which showed the most active erythropoiesis   The serum from patients under liver treatment did not produce this effect

IV —*Thermostability of the factor*

Pernicious anæmia sera were heated to 56°C for 1–2 hours and cultures of megaloblastic and normoblastic marrows were made in parallel in fresh and heated serum   The inhibitory effect was unaltered by this degree of heat showing that the factor is thermostable

TABLE III

The effect of pernicious anæmia sera on normoblastic marrows
(80% serum and 20% Ringer solution)

No of exp	Megaloblasts/100 nucleated red cells		
	Initial count	72 hr cultures in	
		Normal serum	P A serum
N6	0 0	0 0	4 0
N9	0 0	0 0	6 0
N8	0 0	0 0	8 5
N11	0 0	0 0	1 0
N71	0 0	0 0	28 0*
N73	0 0	0 0	1 0
N74	0 0	0 0	7 0
N75	0 0	0 0	3 0

* Counted by five independent observers

V —Presence of the factor in the cerebrospinal fluid

The possible presence of the inhibitory factor in cerebrospinal fluid of patients with untreated pernicious anæmia was next investigated   (The suggestion of using cerebrospinal fluid was made by Dr C C Ungley) The reasons for using cerebrospinal fluid were that the low protein content might make further attempts to isolate the factor easier, and that it had been found technically difficult to obtain suitable serum ultrafiltrates which were both sterile and sufficient in amount

As may be seen in Tables IV and V normal cerebrospinal fluid behaves like normal serum, and pernicious anæmia cerebrospinal fluid shows a positive inhibitory effect

TABLE IV

The effect of cerebrospinal fluid from pernicious anæmia patients, on normoblastic and megaloblastic marrows

No of exp	Megaloblasts/100 nucleated red cells				
	Initial count	72 hr cultures in			
		Normal		P.A	
		Serum	C S F	Serum	C.S.F
N73	0 0	0 0	0 0	14 0	4 0
N75	0 0	0 0	0·0	8 0	2 0
N76	35 0	5 0	0 0	24 0	17 0

TABLE V

Dilution experiments with cerebrospinal fluid from pernicious anæmia patients, on megaloblastic marrows

No of exp	Megaloblasts/100 nucleated red cells			
	Initial count	72 hr cultures in		
		90% P.A. serum + 10% Ringer solution	20% P A serum	
			+ 80% Ringer	+ 80% P.A. C S F
N76	35 0	24 0	7 0	17 0
N77	18 4	23 0	17 0	29 5*

* Counted by three independent observers

VI — *The effect of antipernicious anæmia factors in vitro*

(a) *Folic acid* Cultures of megaloblastic marrows were set up in parallel with and without the addition of folic acid (Folvite, Lederle 2 0 μg /ml medium) both in normal and in pernicious anæmia sera (Table VI) The results show that folic acid had a significant ripening effect on megaloblasts in both media

(b) *Liver extract* Similar experiments were made adding liver extract (Pernæmon forte, Organon, 0 03 ml /ml medium) to the cultures in place of the folic acid The liver was found to have a similar direct ripening effect (Table VII)

(c) *Vitamin B_{12}* The effect of two preparations of vitamin B_{12} was assessed (Cobione, Merck and Cytamen, Glaxo, 0 1 μg /ml medium) The results are in striking contrast to those obtained with folic acid or liver extract There was clearly no significant ripening effect of the vitamin B_{12} on megaloblasts *in vitro* in this concentration, neither did it inhibit the development of megaloblasts from normoblasts (Table VIII)

DISCUSSION

The accumulated evidence from these experiments points to the presence of an inhibitory factor in the serum and cerebrospinal fluid of patients with pernicious anæmia in relapse There is no evidence that this is an abnormal factor , it may be a physiological factor the effect of which is normally overcome by a hæmopoietic factor The concept that pernicious anæmia is not merely a deficiency disease but is due also to the presence of an inhibitor or " toxin " in the organism is supported by some of the earlier experimental work on pernicious anæmia (1, 3, 4, 5, 9)

The experiments in which normoblastic marrows became megaloblastic *in vitro* strongly suggest that the megaloblasts were derived from normoblasts For example in an originally normoblastic marrow, where the predominant

TABLE VI

The effect of folic acid in vitro on megaloblastic marrows

No of exp	Megaloblasts/100 nucleated red cells		
	Initial count	48 hr cultures in	
		Normal serum	Normal serum + folic acid
B3	50 2	15 5	4 0
B4	23 5	12 5	4 5*
B5	41 5	9 5	5 0
B6	79·0	12 0	1 0*
B7	37 5	16 5	8 5
		48 hr cultures in	
		P.A serum	P.A serum + folic acid
B5	41 5	16 5	12 0
B6	79 0	36 0	13 0
B7a	37 5	36 5	30 5
B27	23 5	4 0	0 0
N66a	16 0	20 0	9 5
N66b	16 0	5 0	0 0
N67	46 7	46 6	29 6†
N69	46 0	23 0	14 0
N70	31 5	38 0	19 0
N76	35 0	24 0	5 0
N78	37 0	28 3	10 0‡

* Counted by two independent observers
† Counted by five independent observers
‡ Counted by three independent observers

TABLE VII

The effect of liver extract in vitro

No of exp	Megaloblasts/100 nucleated red cells		
	Initial count	48 hr cultures in	
		P.A serum	P.A serum + liver
B7a	37 5	36 5	15 0
B7b	37 5	27 0	15 5
B8	7 5	7·0	1·0

TABLE VIII

The effect of vitamin B_{12} in vitro

No of exp	Megaloblasts/100 nucleated red cells			
		48 hr cultures in		
	Initial count	P.A serum	P.A. serum + B_{12}	P.A serum + folic acid
N66	16 0	20 0*	14 0	9 5
N67	46 7	46 6*	43 0	29 5
N69	46 0	23 0	27 0	14 0
N70	31 5	38 0†	41 0	19 0
N76	35 0	24 0	14 0	5 0
N78	37 0	28 5	24 5	17 0
N73	0 0	3 0	2 0	
N74	0 0	7 0	4 0	
N75	0 0	2 0	5 0	
N79a	14 0	46 0	44 0	
N79b	14 0	46 0‡	42 0	

* Counted by five independent observers
† Counted by three independent observers
‡ Counted by three independent observers

form of nucleated red cell was the polychromatic normoblast, during the culture in pernicious anæmia serum 28% megaloblasts developed   Numerous transitional forms from normoblasts to megaloblasts were seen and there was no indication from the mitotic index that this large number of megaloblasts had developed from a particular "young" cell form

This would imply that in the pernicious anæmia culture medium there is a change in the metabolism of the normoblasts which results, morphologically, in the megaloblastic form and, functionally, in a slower maturation

There is at present insufficient evidence for the incorporation of these findings into any comprehensive scheme of erythropoiesis   Nevertheless, it is important at every stage in experimental work to put forward a provisional hypothesis which may form the basis of later observations   A hypothesis which is compatible with the results so far obtained is outlined in Fig 2   The mechanism of development of megaloblastic anæmias according to this scheme could be as follows

(a) Folic acid, which is possibly a general growth factor, acts directly on the cells in vitro and acts in vivo without the help of liver factor or vitamin B_{12} Folic acid deficiency produces megaloblastic anæmias which respond only to folic acid   So far as is known no megaloblasts are resistant to folic acid , even in anæmias of complex origin where the hæmoglobin synthesis is impaired by other factors, the megaloblastic marrow returns to normoblastic marrow by treatment with folic acid although this is not necessarily followed by a satisfactory rise in hæmoglobin level

(*b*) The activity of the folic acid normally present is inhibited by a factor present in the serum and cerebrospinal fluid   It is suggested that the effect of this inhibitory factor is normally counteracted by the active liver principle (hæmopoietic factor)   If hæmopoietic factor is absent, *e g*, in pernicious anæmia, the effect of the inhibition becomes evident   It may, however, be overcome by mass action with relatively large doses of folic acid

(*c*) The experimental findings indicate that liver and a normal serum contain a hæmopoietic factor which is not identical with crystalline B_{12} It is possible that the B_{12} molecule acts as a coenzyme to an enzyme system and has to be transformed by the organism to the active hæmopoietic factor Either B_{12} or the hæmopoietic factor is concerned with the normal function of the central nervous system

(*d*) The B_{12} content of the organism depends on the intake in the food, intestinal synthesis and absorption and the presence of the "intrinsic factor"

Vitamin B_{12} Vitamin B_{12} in intestine   Intestinal
in serum   + intrinsic factor   milieu

Folic acid

Hæmopoietic factor   *counteracts*   Inhibitor
(also C N S factor)   *the action of*

" Nuclear structure "
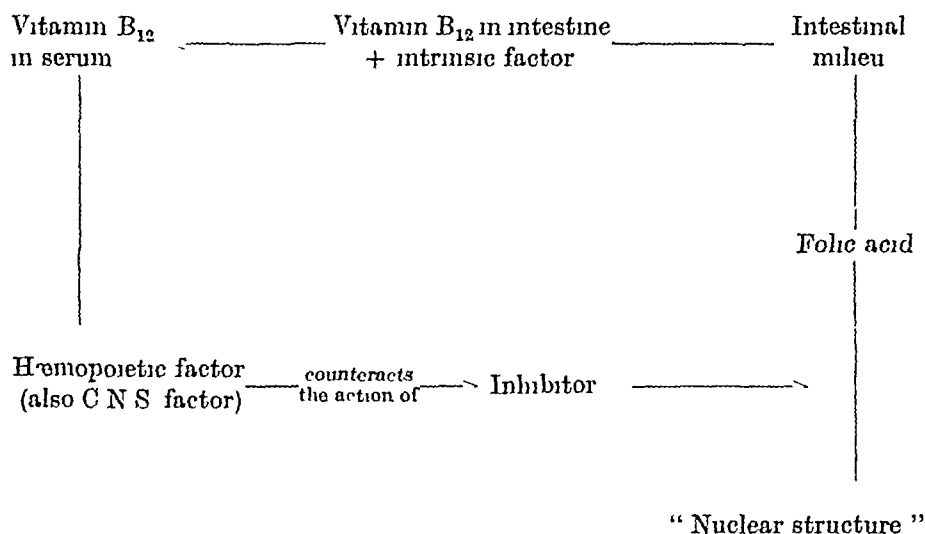
Fig 2   A tentative scheme for the interrelationship of factors controlling normoblastic erythropoiesis

The pathological mechanism of pernicious anæmia according to this hypothesis would be that lack of intrinsic factor causes a deficient absorption of vitamin B_{12} from the intestine   Lack of B_{12} results in a disturbance in the metabolism of the central nervous system and the failure to counteract the effect of an inhibitory factor in the serum   This will result in degenerative changes in the central nervous system and an inhibition of the action of the normal folic acid level   The latter can be overcome by mass action of folic

acid   This will not influence the central nervous degeneration which will continue to progress   Parenteral administration of crystalline B_{12} or liver alone however, will restore normal erythropoiesis and arrest the central nervous changes

SUMMARY

1   Evidence is presented for the presence of a factor in the serum of patients with untreated pernicious anæmia which inhibits the ripening of megaloblasts to normoblasts in bone marrow cultures

2   This factor will cause the transformation of normoblasts to megaloblasts in cultures of originally normoblastic marrows

3   The factor is thermostable

4   It is present in the cerebrospinal fluid of patients in relapse

5   Folic acid and liver extract will overcome the effect of the inhibitory factor *in vitro*

6   Vitamin B_{12} has no influence on the inhibition *in vitro*

7   A scheme is presented for the interrelationship between the inhibitory factor and hæmopoietic principles and a possible mechanism of megaloblastic anæmias is discussed

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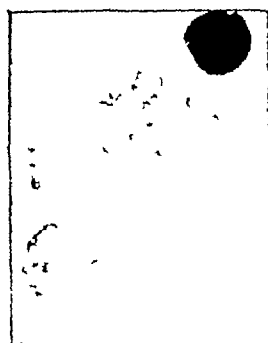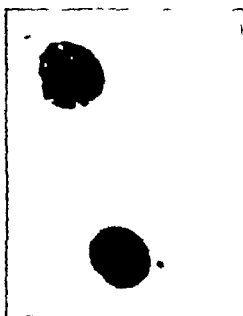
MEGALOBLASTIC MARROW INITIAL STATE
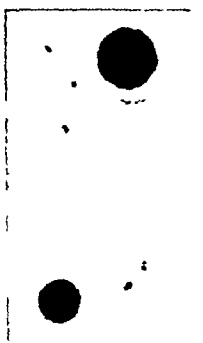
48hr CULTURE IN CONCENTR NORMAL SERUM
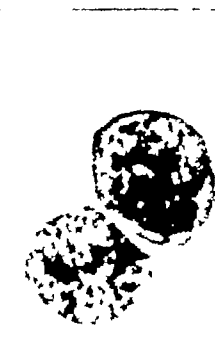
48hr CULTURE IN CONCENTR PERNICIOUS ANAEMIA SERUM
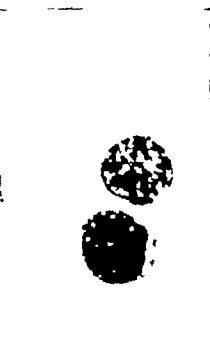
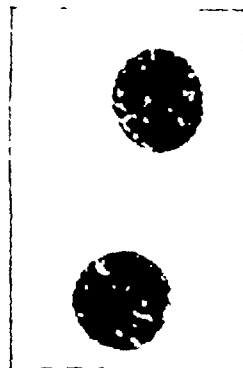48hr CULTURE IN DILUTED PERNICIOUS ANAEMIA SERUM

NORMOBLASTIC MARROW INITIAL STATE
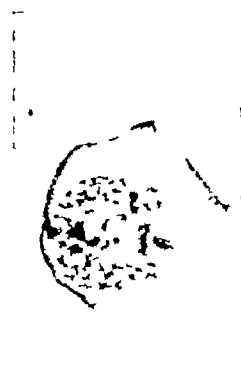
72hr CULTURE IN CONCENTR NORMAL SERUM

72hr CULTURE IN CONCENTR PERNICIOUS ANAEMIA SERUM

NORMOBLASTIC MARROW INITIAL STATE

72hr CULTURE IN CONCENTR NORMAL SERUM

72hr CULTURE IN CONCENTR PERNICIOUS ANAEMIA SERUM

OBSERVATIONS ON THE STRUCTURE OF CLUBBED FINGERS

By R R H LOVELL *

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CLUBBING of the fingers has been recognised for centuries It has well known associations with a variety of apparently unrelated disorders and may occur as an isolated hereditary deformity Yet not only has the pathogenesis of clubbing remained a matter of speculation but there is little agreement on the nature of the structural change in the finger ends which accounts for their appearance

The purpose of this investigation has been to determine the structural basis for the appearance of clubbing The observations fall into two groups namely those made during life and those made on fingers obtained at necropsy

PART 1 CLINICAL OBSERVATIONS

The physical signs

Clubbed fingers associated with a variety of diseases were examined to analyse the features which distinguish them from normal fingers These features may be considered as (a) those which are constantly found and (b) those which, though common, are not constant The former reflect the essential structural changes and it is on their recognition that the clinical diagnosis of clubbing depends

(a) *Constant changes* 1 The shape of the distal segment of the finger is altered Though this was noted by Pigeaux (1) and emphasised by Lovibond (2) clubbing is still sometimes incorrectly described as symmetrical enlargement of the distal finger segment When the finger is viewed laterally, there is on the dorsal contour a flattening of the normal obtuse angle formed by the proximal part of the nail with the plane of the soft tissue covering its root This alteration in shape contrasts with the

shape of the finger showing symmetrical enlargement of the distal segment, in which the contours on the dorsum remain comparable to those of a normal finger   An unusual case of hypertrophy of the distal segment of one index finger, the cause of which was not determined, illustrates the difference   (Fig 1)
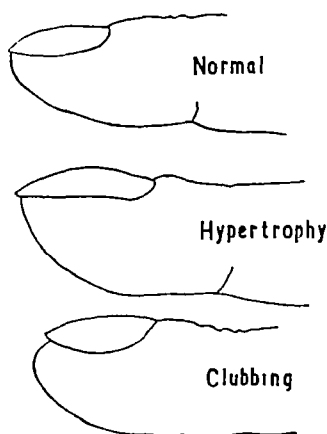

Fig 1   Tracings from photographs   The normal and hypertrophied fingers are index fingers of the same subject, referred to in the text

2   The texture of the tissues is altered   This is apparent on palpating over the nail bed where fluctuation is greater than in normal fingers   The nail plate is unusually mobile both laterally and longitudinally

3   The distal segment is increased in volume   This change, which is often obvious on inspection, was confirmed by measurement in two cases in which the reduction of clubbing, induced by treatment of the associated disease, was accompanied by reductions in distal segment volumes of 0 93 ml (12%) and 0 43 ml (10%) respectively   (Cases 2 and 3 described later)

4   The shape of the nail, which varies greatly in healthy people, usually shows increased curvature in one or both planes   The form of the nail is a manifestation of earlier events in the nail bed and root   Thus an illness or period of mental stress may be manifest by distally convex transverse ridges in the nails, known as Beau's lines, which grow out and leave in time healthy looking nails   Nails of fingers developing clubbing rarely indicate a sudden change of this sort , usually they gradually assume a curved deformity   The remission of clubbing however, if rapidly induced by the relief of the associated illness, may be manifest in the nail plate by a distinct line of demarkation between the abnormally curved and the succeeding healthy nail

Case 1 Female, aged 20, suffered from rheumatic carditis and subacute bacterial endocarditis The fingers were moderately clubbed when treatment with Penicillin was started on the 6th November, 1947 On 2nd February, 1948, the fingers were no longer clubbed but the nail plates showed in varying degree the angular deformity illustrated in the right thumb in Fig 5 (*a*) Transverse curved ridges and grooves were present at the angulations Measurement of a nail plate showed the ridge to be about 10 mm from the nail root, representing some twelve weeks growth at a normal rate The Penicillin therapy was actually started 12½ weeks before On 16th April, 1948, the patient was well and the fingers appeared normal (Fig 5 (*b*))

A similar sequence of changes has since been sought and observed in other patients with subacute bacterial endocarditis cured with Penicillin Such cases provide direct evidence of change affecting the nail matrix in clubbing

(*b*) *Inconstant changes* These include enlargement of the pulp of the finger, flushing of the skin round the nail and longitudinal striations in the nail which some patients claim grows faster than usual Unusual glossiness of the skin over the nail root is often apparent, the fine fissures ordinarily present disappearing for 5 to 7 mm proximal to the eponychium These manifestations of clubbing may be variable because they are not essential features or because they are modified by either the patient's occupation or the manicuring to which he submits his fingers

The sequence of changes

The earliest signs of clubbing are found in the region of the nail bed Increased fluctuation in this region, though not susceptible to exact measurement, occurs before any obvious alteration in volume or contour It may be accompanied by loss of fissuring in the skin of the eponychial fold and flushing of the skin in this region mentioned already Since it is usually much easier to see the early changes in the dorsal contour than to feel the increased fluctuation, the former constitutes the first incontrovertible sign of clubbing

Classification

An entirely satisfactory classification of degrees of clubbing was not attained and it was thought that the appearances of clubbed fingers depended to some extent on the initial form of the distal finger segments In describing the deformity the following degrees of clubbing were denoted —

Slight Increased fluctuation over the nail bed Flattening of the dorsal contour

Moderate Increased fluctuation over the nail bed Flattening of the dorsal contour Appreciable enlargement of the pulp Increased curving of the nail

Marked   Increased fluctuation over the nail bed   Flattening of the
dorsal contour   Conspicuous enlargement of the pulp   Increased
curving of the nail

Gross   Conspicuous fluctuation over the nail bed   Flattening of the
dorsal contour with raising of the whole nail bed region   Con-
spicuous enlargement of the pulp   Conspicuously increased curving
of the nail

This analysis of the features which distinguish clubbed from normal
fingers and those otherwise deformed indicates the nail bed as the site of
early and constant changes

Structural changes in clubbed fingers

Alteration in any of the tissues composing the distal finger segment
might in theory account for the shape of clubbed fingers   Thus the distal
segment may be considered in terms of the skin and its appendages, bone,
tissue fluid, blood, blood vessels and interstitial tissue

In the skin and its appendages no alteration was found, apart from
that of the nail plate already described, which might affect the shape of the
finger

Bone changes in the terminal phalanges have been studied radiologically
Locke (3) found that 22 of a series of 39 cases of clubbing showed no bone
changes   In those with changes there was an irregular hypertrophy of the
distal half of the terminal phalanges   The phalanges clearly do not
ordinarily contribute to the changes in the shape of the fingers

Œdema of the distal segment is difficult to demonstrate clinically even
in such cases as superior vena caval obstruction when the arms and hands
are swollen   Increase in tissue fluid in such cases is manifest in the parts
where connective tissue is loose, but the firm fibrous structure of the distal
segment prevents obvious accumulation of fluid   Such cases do not develop
the appearances of clubbing   Compression of the fingers with an elastic
bandage in a patient with osteoarthropy associated with bronchial carcinoma,
whose hands and feet were œdematous, failed to alter the shape of the
clubbing though the constricting bandage reduced the œdema in the more
proximal finger segments   Such evidence is against œdema alone being the
basis for the shape of the fingers

If the shape in clubbing is due to dilatation or proliferation of blood
vessels alone, it should be possible by compressing the finger firmly with
an elastic bandage to empty the blood vessels and restore a normal shape
In clubbing associated with bronchopulmonary diseases, subacute bacterial
endocarditis and ulcerative colitis such compression has failed appreciably
to alter the shape of the finger   In some cases associated with cyanotic
congenital heart disease an alteration in shape was induced by this method,

though the fingers remained indisputably clubbed   The alteration in shape was accompanied by a diminution in tension in the tissues at the base of the nail, a change which could not be detected with certainty in other cases

These observations suggest that the shape of clubbed fingers is essentially dependent on an increase in tissue, though in addition an increased vascularity is indicated in some cases associated with cyanotic congenital heart disease   The region of the nail bed is again emphasised as the site of prominent change

The nature of the increased volume in clubbed fingers

The demonstration that an increased vascularity in clubbing does not account entirely for the shape of the finger does not preclude the presence of such a change   At least a functional vascular change, an increased blood flow, has been demonstrated (4)   An attempt was therefore made to compare the blood volumes of the distal segments of clubbed fingers and normal fingers

Method   To attain uniformity, middle fingers were used   The distal segment volume was first measured by the method described by Lennard-Jones* (5)   The hand was then raised above the head until it paled, when an elastic bandage about 1 cm wide was wound firmly over the finger, starting at the tip   The distal part of the bandage was unwound and the bandage made fast at the base of the finger so as to continue to occlude the digital arterial pulses   The distal segment volume was measured once more   In each case the volume recorded was the mean of three measurements   The difference between these volumes was recorded as the reducible finger volume and was expressed as a percentage of the initial finger volume   This percentage reduction in volume was regarded as an index of the blood volume in the distal finger segment   The variations in the measurements of the same fingers on different days were between 0 1 and 0 4 ml for initial volumes, between 0 02 and 0 26 ml for reducible volumes and between 0 3 and 7 0 for percentage reductions in volume

Results   Observations were made on 14 normal subjects, 10 subjects with clubbing associated with bronchopulmonary diseases and 8 subjects with clubbing associated with cyanotic congenital heart diseases   The results are shown in Table I   In Group I the mean initial volume was 4 97 ml (S D 1 96), in Group II 6 66 ml (S D 1 47) and in Group III 4 55 ml (S D 2 14)

The mean percentage reduction in volume in Group I was 4 34 (S D 1 98), in Group II 5 03 (S D 1 93) and in Group III 8 48 (S D 3 14)   In this respect Group II does not differ significantly from Group I (P = 0 4) but the mean percentage reduction in volume in Group III is significantly greater than in Group I (P = 0 01) and Group II (P = 0 02)

Group III —Cyanotic congenital heart disease clubbed fingers

Group	Number	Sex	Disease	Initial volume (ml)	Compressed volume (ml)	Reducible volume (ml)	Percentage reduction	Degree of clubbing
I	1	M	Healthy	5 60	5 28	0 34	6 07	None
	2	M	Healthy	5 50	5 33	0 17	3 09	None
	3	M	Healthy	5 43	5 26	0 17	3 13	None
	4	M	Duodenal ulcer	5 62	5 33	0 29	5 16	None
	5	M	Gastric ulcer	7 18	6 83	0 35	4 87	None
	6	M	Functional dyspepsia	7 90	7 62	0 28	3 54	None
	7	M	Healthy	5 70	5 28	0 42	7 36	None
	8	M	Diabetes	6 22	6 17	0 05	0 08	None
	9	M	Healthy	4 77	4 58	0 19	3 98	None
	10	M	Healthy	6 22	5 87	0 35	5 62	None
	11	F	Torticollis	2 34	2 16	0 18	7 70	None
	12	F	Healthy	1 54	1 47	0 07	4 55	None
	13	M	Fractured tibia	2 73	2 68	0 05	1 83	None
	14	M	Primary tuberculosis	2 95	2 86	0 09	3 05	None
			MEAN	4 97			4 34	
			STANDARD DEVIATION	1 96			1 98	
II	15	M	Bronchiectasis	3 93	3 73	0 20	5 08	Marked
	16	M	Bronchial ca	9 05	8 70	0 35	3 86	Gross
	17	M	Bronchial ca	7 62	7 27	0 35	4 50	Gross
	18	M	Bronchial ca	5 67	5 43	0 24	4 23	Marked
	19	M	Suppurative pneumonia	7 48	7 23	0 25	3 34	Marked
	20	M	Emphysema	5 88	5 52	0 36	6 12	Marked
	21	M	Emphysema	6 88	6 32	0 58	8 13	Marked
	22	M	Bronchial ca	5 42	5 03	0 39	7 17	Marked
	23	M	Bronchial ca	6 92	6 52	0 40	5 78	Marked
	24	M	Suppurative pneumonia	7 73	7 57	0 16	2 06	Gross
			MEAN	6 66			5 03	
			STANDARD DEVIATION	1 47			1 93	
III	25	F	Congenital heart	2 42	2 07	0 35	14 46	Gross
	26	F	"	2 60	2 48	0 21	7 80	Gross
	27	M	"	2 82	2 52	0 30	10 63	Gross
	28	F	"	5 26	5 05	0 21	3 99	Marked
	29	M	"	8 98	8 30	0 68	7 57	Gross
	30	M	"	5 57	5 11	0 46	8 25	Gross
	31	F	"	5 02	4 67	0 35	6 97	Gross
	32	F	"	3 07	3 37	0 30	8 20	Gross
			MEAN	4 55			8 48	
			STANDARD DEVIATION	2 14			3 14	

In drawing conclusions from these figures it is necessary to consider the possibility of a correlation between the initial finger volumes and the percentage reductions in volume (Fig 2) Owing to the relatively small stature of the congenital heart cases, which show the greatest reductions in volume, their finger volumes tend to be small and this might be held to account for their greater reducibility Calculation shows no such correlation
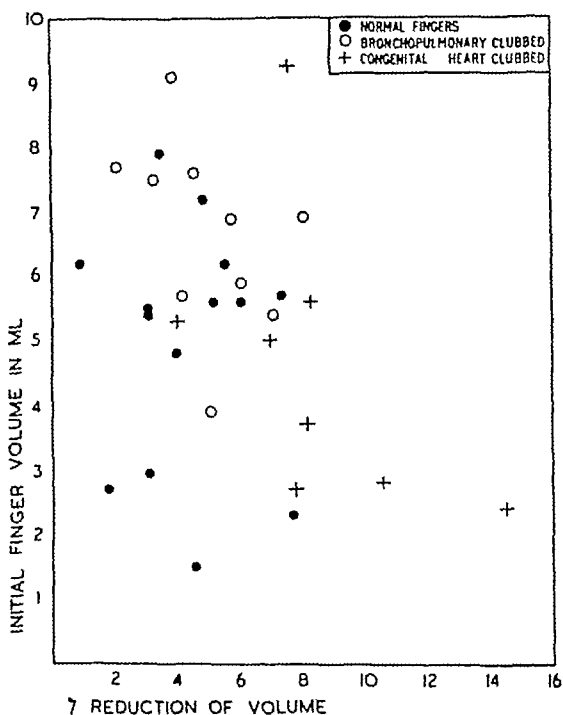

Fig 2

These measurements indicate no appreciable difference in blood volumes in the distal segments of normal fingers and clubbed fingers associated with bronchopulmonary diseases as a whole The blood volumes of the distal segments of clubbed fingers associated with cyanotic congenital heart diseases are relatively increased A considerable range of blood volumes is shown in each group and some of this scatter may be due to inaccuracy in the method of measurement

It is not possible with these figures to test a correlation between distal segment blood volumes and degrees of clubbing, for the latter are not indicated by the initial volumes of the distal segments All the clubbed fingers measured were markedly clubbed, numbers 16, 17 and 24 in Group II were outstandingly deformed and it is noted that the percentage reductions

in volume in these cases were below the average for the group   This finding accords with the observation that even the most grossly clubbed fingers associated with bronchopulmonary diseases are not altered in appearance by compression

Further evidence of the absence of measurably increased blood volume in a bronchopulmonary case was gained by successive measurements of a finger in which the clubbing disappeared following treatment of the associated disease

Case 2   Male, aged 49, suffered from chronic suppurative pneumonia, which showed rapid clinical and radiological regression under treatment with Penicillin   Measurements made on the right middle finger were as follows —

		Initial Vol	Com-pressed Vol	Reducible Vol	% Reduction
22 10 48	Marked clubbing	7 48 ml	7 23 ml	0 25 ml	3 34
8 11 48	Slight clubbing	6 55 ml	6 34 ml	0 21 ml	3 20

Thus the regression of clubbing was associated with reduction in distal segment volume of 0 93 ml (12%) but there was no significant change in the reducible finger volume such as might have been expected had the clubbing been associated with vascular engorgement   In fact the compressed finger volume decreased with the regression of the clubbing which suggests an actual decrease in tissue substance in the distal finger segment

Comparable results were observed in a case of subacute bacterial endocarditis

Case 3   Female, aged 30, suffered from rheumatic carditis, and subacute bacterial endocarditis which was cured by Penicillin   Measurements made on the right middle finger were as follows —

		Initial Vol	Com-pressed Vol	Reducible Vol	% Reduction
22 10 48	Moderate clubbing	4 18 ml	4 05 ml	0 13 ml	3 12
21 12 48	Normal finger	3 75 ml	3 60 ml	0 15 ml	4 00

Again, the diminution in distal segment volume of 0 43 ml (10%) was not associated with significant change in the reducible finger volume

These volumetric studies further confirmed the conclusions suggested by clinical observation, that while a fundamental change in clubbed fingers is an increase in interstitial tissue in the fingers, an additional increase in blood volume occurs in clubbing associated with cyanotic congenital heart disease

PART 2   MORBID ANATOMICAL STUDIES

Despite the frequency with which clubbed fingers are seen, recorded observations on their structure are few   In fact the published observations do not truly reflect the interest in this subject   Sections made by the usual methods look remarkably normal, an observation which must often have been deemed unworthy of record   Furthermore, clubbing becomes less obvious after death   This is due to the shrinking of the pulp of the finger causing infolding of the skin, a change which is also seen in normal fingers The essential alteration in contour on the dorsum of the clubbed finger remains unchanged, as does the sense of increased fluctuation over the nail bed

In his review of the publications on clubbing, Mendlowitz (6) summarised the pathological changes reported   These vary with different authors and include changes in some or almost all of the tissues of the distal finger segment   Many observations were based on naked eye appearances alone, sometimes without reference to normal appearances or changes which might have been associated with terminal venous congestion   The study of such publications suggested that whatever pathological changes may be present in clubbing they are not strikingly demonstrated by ordinary histological methods, and this proved to be the case   Observations were therefore made by methods especially designed to demonstrate the vascular anatomy of the distal finger segment, so that the structural basis for the distinction between clubbing associated with bronchopulmonary and cyanotic congenital heart diseases might be determined and other vascular changes sought

Intra-vascular staining

Grant (7) and Grant and Bland (8) described their use of a method of intravascular fixation and staining which proved useful in studying arteriovenous anastomoses in fingers and elsewhere   The essential features of this method are (a) fixation of the finger with the blood vessels dilated by means of arterial injection of formol saline under pressure, the venous outlet being almost completely occluded, and (b) staining of the vessel walls by the arterial injection of Ehrlich's acid hæmatoxylin   When thick sections of such specimens are examined in order to study the minute vascular anatomy the extent of vascular filling is apparent and the distinction between arteries and vein manifest by the appearances of the stained vessel walls   Arteriovenous anastomoses are also clearly revealed   The difficulty in assessing the relative sizes of blood vessels inherent in ordinary histological methods is also overcome, the blood vessels all appearing dilated   Grant (7) noted in preparations of the rabbit's ear made by this method that the diameter of the anastomoses agreed well with their diameter measured when maximally dilated during life, which suggests that the method induces no great vascular distortion

TABLE II

Fingers examined by intra arterial fixation and staining

Finger	Clubbing	Disease	Age	Sex	Thickness of nail bed in mm	Mean diameter of nail bed veins in μ	Mean diameter of main nail bed arteries in μ
GB	None	Subarachnoid hæmorrhage	58	F	1 5	63	177
GF	None	Carcinoma of rectum	45	F	2		
G	Gross	Cyanotic congenital heart	15	M	3	105	208
GC	Moderate	Carcinoma of bronchus	50	M	3	58	
GD	Marked	Bronchiectasis	45	F	3		
GE	Gross	Bronchiectasis	42	F	3 5	73	257
GG	Gross	Bronchiectasis	17	M	2 5	56	235

Method A canula having been tied into one digital artery, the technique of injection described by Grant (7) for fixation and staining was followed Immediately after the excess hæmatoxylin was washed out the finger was frozen in a solid carbon dioxide-methylated spirit mixture for a few minutes, rapidly transferred to a vice and the distal finger segment was then sawn transversely into blocks about 3 mm thick When thawed the bone was dissected from each block and sections 200μ thick were cut with a freezing microtome The sections were decolourised under the microscope, blued, dehydrated with alcohol and mounted in Canada Balsam Experience with this method showed that it was desirable to cut and mount sections immediately after staining as storage of sections or blocks often led to loss or spread of the stain In the present studies however such uninterrupted periods of work were impossible and in many cases sections were re-stained with hæmatoxylin before mounting The stain was not then confined to the blood vessels but satisfactory results were obtained and it was also found that light counterstaining with eosin did not obscure the vascular anatomy

Two normal fingers and five clubbed fingers were studied by this method The clubbed fingers consisted of one from a case of cyanotic congenital heart disease and four from cases of bronchopulmonary diseases (Table II)

Results Sections cut through comparable parts of the distal finger segments were examined In the five clubbed fingers the nail beds were thicker than in the two normal fingers and this appeared due to an increase in connective tissue The fibrous capsule enclosing the finger pulp was also thicker in the clubbed fingers though this was less obvious than the change in the nail beds There was no difference in the appearance of sweat glands, fat deposits, Paccinian corpuscles and epithelium

The clubbed finger from the case of cyanotic congenital heart disease differed from the normal and other clubbed fingers in that the vessels forming the venous plexuses in the nail bed were greatly increased in calibre (Fig 4) A similar, though less striking difference was apparent in the equivalent vessels of the skin over the pulp The deeper plexuses (third and fourth venous plexuses of Spalteholz) were affected rather than the more superficial subpapillary plexuses (first and second venous plexuses of Spalteholz) In one normal finger and four of the clubbed fingers it was possible to measure the diameters of the vessels forming the skin venous plexuses in the nail beds In each case, nine serial sections 200μ thick were taken from the finger immediately distal to the lunula Using a projecting microscope, the diameters of these veins which appeared transversely cut across in the sections were measured The results are recorded in Table III and illustrated in Fig 3 These measurements show clearly the large mean size of the nail bed veins in the clubbed finger from a patient with cyanotic

TABLE III

Diameter of veins counted in nine serial sections through each nail bed

Diameter in μ	Number of veins counted				
	Normal	Clubbed ca bronchus	Clubbed bronchiectasis	Clubbed bronchiectasis	Clubbed congenital heart
20	35	38	25	20	7
40	88	110	94	124	48
60	74	52	72	49	69
80	28	29	64	8	71
100	12	6	25	5	59
120	3		13	1	44
140	3		6		31
160			1		24
180					12
200					5
220					3
240					1
260					1
TOTAL NUMBER COUNTED	243	235	300	207	375
MEAN NUMBER PER SECTION	27	26	33	23	42
MEAN DIAMETER	63 μ	58 μ	73 μ	56 μ	105 μ
STANDARD DEVIATION	30	22	43	19	48

congenital heart disease as compared with the normal and bronchopulmonary clubbed specimens No conclusion could be drawn from the apparent increase in the number of veins cut across in the congenital heart specimen, as this could be accounted for as well by increased tortuosity as by absolute increase in number of the veins

The arteries were also studied in some of these specimens Neither the main cutaneous arteries nor their branches forming the cutaneous arterial network appeared to differ in number in normal and clubbed fingers In the clubbed specimens the main nail bed arteries appeared larger than

in the normal ones and such measurements as were possible supported this conclusion (Table II)   In sections from comparable parts of each clubbed finger there were arteries of this order with internal diameters double those of the largest comparable arteries in the normal fingers

The results of these observations accord well with the conclusions reached by the clinical studies, illustrating the anatomical basis for the increase in blood volume in clubbed fingers associated with cyanotic congenital heart disease
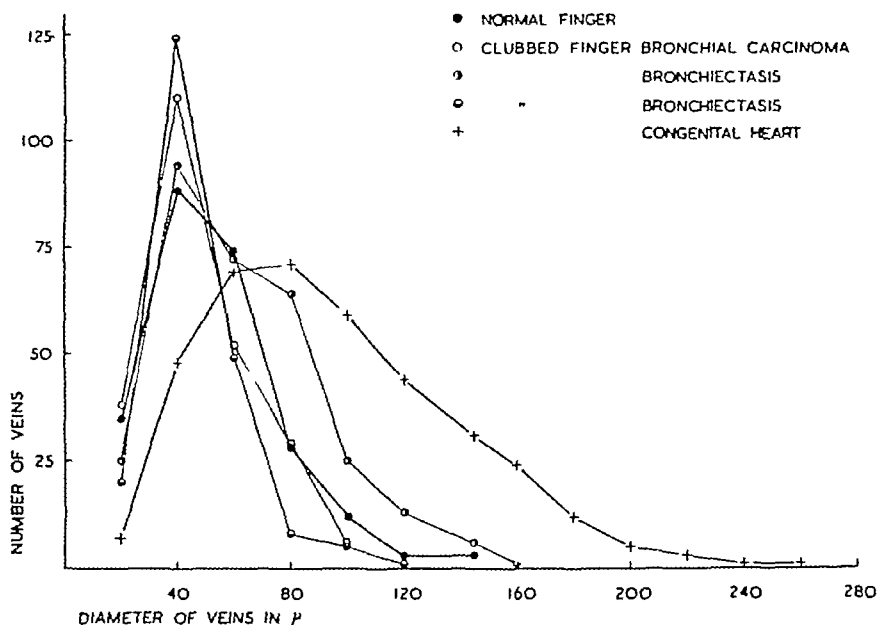
Fig 3   Shows the frequency of distribution of veins of different diameter in five fingers   The diameter of the veins was measured as described in the text

Neoprene studies

Though the intravascular staining method proved useful for examining the minute skin vessels and particularly the veins, it did not reveal the general arterial arrangement in the distal finger segment , studies were therefore made by the intra-arterial injection of neoprene

Casts of the digital arterial tree were made in two normal fingers by intra-arterial neoprene injection and subsequent digestion of tissue, using the method described by Trueta and others (9) for the kidney   The small blood vessels thus reproduced were found to be so complex that they could not satisfactorily be analysed   Studies were then made by the following method, combining neoprene injection with the Spalteholz method of clearing large specimens

Method   In injecting the neoprene the procedure described by Trueta and others (9) was closely followed   Before releasing the injection pressure the base of the finger was firmly ligated and it was then immersed in a freezing mixture of solid carbon dioxide and methylated spirit for about five minutes   This resulted in the irreversible change of the neoprene from liquid to a solid state   The finger was rapidly transferred to a vice and sections 2-5 mm thick were cut transversely or longitudinally with a fine bladed hacksaw , the sections were fixed in 10% formol saline for 24 hours, dehydrated by passage through alcohol and transferred to methyl salicylate in which they were cleared, stored and subsequently mounted for examination with a binocular microscope or hand lens

Four fingers of normal appearance and three clubbed fingers were examined by this method   There were differences in the extent of the neoprene filling between the normal and the clubbed fingers   Solid medium injection methods are however of limited value in comparative studies of vascular anatomy   Unless the injected specimens are ultimately submitted to histological examination it is difficult with certainty to identify the vessels filled as arterial or venous , the risk of error due to lymphatic filling is ever present , a lack of filling cannot be accepted as evidence of absence of a blood vessel or of vascular obstruction   Having regard for these errors inherent in the method, the differences between the normal and clubbed fingers cannot be regarded as significant   Nevertheless the technique proved useful in studying the digital arterial anatomy (Fig 6) and for that reason it is recorded

Normal vascular anatomy   The vascular arrangements in the distal finger segment are sufficiently complex and important to warrant comment The following is partly based on the study of eight normal fingers by methods described above

The distal finger segment has a remarkably profuse arterial supply as compared with the more proximal parts (Fig 6), and the main digital arteries diminish but little in calibre until the distal segment is reached   The proximal part of the nail bed derives its arterial supply from an arch passing dorsally over the base of the distal phalanx, while the most distal part is supplied with branches from the main pulp vessels   There is free anastomosis between major branches of the digital arteries in the distal segment

The venous arrangement over the pulp of the finger is the same as that in thin skin elsewhere , the venous plexuses are extensive but extend to no great depth   In the nail bed the venous plexuses extend to greater depth, the four plexuses named by Spalteholz being clearly defined   (Fig 4 (*a*))   The deep third and fourth plexuses, as in the pulp, are intimately connected to tortuous collecting veins which drain the arteriovenous anastomoses, whose afferent limbs arise from branches of the main cutaneous

arteries   Under the nail root the venous radicles are so numerous and complex that they form an almost angiomatous mass (Fig 7), this being the reservoir to be filled when the arteriovenous anastomoses are open

The arteriovenous anastomoses described by Hoyer (10) have been studied by Grant and Bland (8) who found that the nail bed contains some five hundred anastomoses per square centimetre surface area   This is more than twice as many as are present in the same surface area elsewhere in the distal segment or hand   Though the figures reported by Popoff (11) are much lower than those of Grant and Bland, he too found the greatest density of anastomoses in the nail bed

<div align="center">DISCUSSION</div>

The essential structural difference between normal and clubbed fingers consists in an increase in connective tissue in the distal finger segment This is demonstrable in sections through the fibrous capsule which encloses the pulp of the finger, but both clinical and morbid anatomical studies show that it is maximal between the nail and the dorsum of the terminal phalanx This selective distribution endows a clubbed finger with its characteristic shape

A further change is found in clubbed fingers associated with cyanotic congenital heart diseases, namely an increase in the volume of the blood in the distal finger segment   This is shown by clinical and volumetric studies, and the anatomical basis is shown by intravascular staining to be a dilatation of the venous plexuses of the skin   This change is also most conspicuous in the nail bed, and while it is not fundamental to the appearance of clubbing it naturally enhances that appearance

The failure to make this distinction between the two types of clubbing accounts for much of the confusion which has existed about the nature of the structural change in the fingers   Hitherto, while clubbed fingers have generally been regarded as a single clinical entity, the conception of their structure has been based on a medley of changes described by various writers who examined single specimens (6)   In fact the distinction between the two types of clubbing is apparent from comparison of some of the earlier descriptions   For instance Variot (12) described the clubbing in a case of cyanotic congenital heart disease   " Microscopic sections of the pulp of the finger show that the venous plexus deep to the skin is markedly dilated as are the capillary loops which pass up in the papillæ   All these vessels and capillaries are filled with red blood cells which indeed form a natural injection mass "   This may be contrasted with the description by Strangeways and Ponder (13) of a markedly clubbed finger associated with " malignant endothelioma " of the lung   " The subcutaneous tissue seems more fibrous and is increased in thickness at the ends of the fingers and toes and thus appears to be the cause of the clubbing "   Histological examination showed " the subcutaneous tissue at the end of the finger perhaps contains

more fibrous tissue than is usually present, but no other change can be made out " Moore (14) reported that he could reduce the clubbing at necropsy, in the case of a child dying with cyanotic congenital heart disease, by cutting into and pressing the clubbed ends, this observation and the microscopic appearance of vascular engorgement led him to conclude that clubbing was chiefly due to engorgement, as indeed it is in such cases This conclusion, however, was not accepted in the light of earlier descriptions of increased connective tissue derived from the study of bronchopulmonary cases (15)

The structural distinction between bronchopulmonary and cyanotic congenital heart types of clubbing does not necessarily imply a different pathogenesis, an increase in connective tissue being common to both types Furthermore, while it is clear from the results of these studies that venous dilatation does not generally occur in the bronchopulmonary group, some of the volumes recorded in Table I and the nail bed vein diameters in finger G E (Table II) suggest that some dilatation may occur in individual cases It is possible that the measurably increased blood volume and venous dilatation in the clubbed fingers of cyanotic congenital heart disease is a manifestation of the great increase in total blood volume and the polycythæmia commonly found in these cases It might, therefore, be expected also to occur in association with some bronchopulmonary diseases where an increase in blood volume is also found

The observation that in clubbed fingers the distal segment branches of the digital arteries are of greater calibre than in normal fingers accords with the findings of Charr and Swenson (16) whose radiological studies on necropsy specimens have been published since this investigation was started They also describe a heavier network of arterioles covering the ungual process in three clubbed fingers than in one normal control An increase in the number of arterioles was not demonstrated in these studies and it is possible that the increased vascularity noted in the radiological studies is due to filling of the skin venous plexuses through the arteriovenous shunts

These observations indicate the anatomical basis for the increased blood flow described in clubbed fingers by Mendlowitz (4) and confirmed by Cross and Wilson (17) It is for consideration whether an increased blood flow alone could account for the selective increase in fibrous tissue which is responsible for the appearance of clubbing Increase in blood flow, except in association with chronic inflammation, is not a recognised precursor of fibrous tissue proliferation, and in clubbing there is no evidence of local inflammatory change The increased flow in clubbing appears to be determined by factors other than local tissue requirements The distal finger segment with its profuse vascular supply and numerous arteriovenous anastomoses is peculiarly adapted to deal with such an increased blood flow A possible mechanism for the increased formation of fibrous tissue is

suggested by consideration of the distribution and action of the arteriovenous anastomoses   Their predominence in the nail bed, where a special venous sump is found (Fig 7) to deal with their effluent, coincides with the site of maximal fibrous tissue formation in clubbing   Furthermore, in spite of an increased blood flow clubbed fingers do not exhibit more conspicuous capillary pulsation than do normal fingers at ordinary room temperatures, such as would be expected if the increased blood flow was subserved by dilatation of the small arterioles in the skin   Thus the increased blood flow in clubbed fingers is probably largely diverted through the arteriovenous anastomoses

The local circulatory changes resulting from the opening of arteriovenous anastomoses of comparable order has been observed directly in the rabbit's ear (7)   A reversal of blood flow was induced in the veins which, when the anastomoses were closed, were tributaries of the potential primary collecting veins   The equivalent tributaries in the nail bed and skin of the finger consist of vessels forming the deep skin plexus, the third and fourth venous plexuses of Spalteholz   A persistently increased blood flow through the arteriovenous anastomoses could thus induce a state of chronic passive congestion in the venous plexuses of the nail bed and skin   Chronic distension of tissue spaces by increased formation of tissue fluid is a suggested precursor of fibrous tissue proliferation (18, 19), which would be anticipated in just that distribution which is found in clubbed fingers

The site and nature of the structural changes in clubbed fingers are thus consistent with the view that clubbing is the result of blood flow through the fingers in excess of the local physiological requiremen's, the manifestations in the distal segment depending on the special local vascular arrangements   Clubbing is in fact due to "forced feeding" (20)   It is by the study of the mechanism of this increased blood flow that the real cause of clubbing should be sought

SUMMARY

1   The appearances which distinguish clubbed from normal fingers have been analysed   The earliest and most constant change is in the nail bed

2   Exsanguination of the finger by an elastic bandage altered the appearance and the tension in the nail bed of clubbed fingers associated with congenital heart disease, but produced no change in normal or other clubbed fingers

3   The distal segment volumes of fourteen normal fingers, ten clubbed fingers associated with bronchopulmonary diseases and eight clubbed fingers associated with cyanotic congenital heart diseases were measured before and after compression with an elastic bandage   The reduction in volume was regarded as an index of the volume of blood in the distal segment

This was significantly greater in the congenital heart group than in the normal and bronchopulmonary groups, which did not differ significantly from one another in this respect

4   In one case of suppurative pneumonia and one of bacterial endocarditis clubbing regressed following cure by Penicillin , in each the distal segment of the finger showed no decrease in reducible finger volume as clubbing diminished

5   It is therefore concluded that the volume of blood in the distal segment of the finger is increased in clubbing due to congenital heart disease but not in clubbing from bronchopulmonary disease   The peculiar shape of the clubbed fingers appears to be due to an increase in tissue especially in the region of the nail bed

6   Autopsy specimens of two normal fingers, one clubbed finger from a case of cyanotic congenital heart disease and four clubbed fingers from cases of bronchopulmonary diseases were examined after the blood vessels had been fixed and stained by means of intra-arterial injections of formalin and hæmatoxylin   Increased fibrous tissue, especially in the nail bed was found in all the clubbed fingers   The skin venous plexuses in the congenital heart specimen were conspicuously dilated, especially in the nail bed

7   The dilatation of the veins in clubbing due to congenital heart disease, but not in bronchopulmonary disease, was confirmed by measurements in fingers from one normal, one congenital heart, and three bronchopulmonary cases   These specimens also revealed an increase in the calibre of the branches of the digital arteries in clubbed fingers

8   In four normal fingers and in three clubbed fingers the digital arteries were injected with neoprene and the tissues subsequently cleared by Spalteholz technique   No differences in vascular filling were observed between the two groups which might not have been attributable to artefacts   The specimens proved useful for the study of the arterial anatomy of the finger

9   The normal vascular anatomy in the distal finger segment as revealed by these methods is briefly described   The nail bed is the site of peculiarly complex vascular arrangements

10   It is concluded that the chief component of clubbing in all the diseases studied is an increase in the connective tissue in the clubbed segment of the finger particularly between the nail and the phalanx   In congenital heart disease an additional component is increased blood volume in the venous plexuses, a feature which may perhaps be correlated with increased total blood volume   The connective tissue increase seems to be due to increased blood flow in excess of local physiological needs and its location in the distal finger segment is attributed to the predominence there of arteriovenous anastomoses

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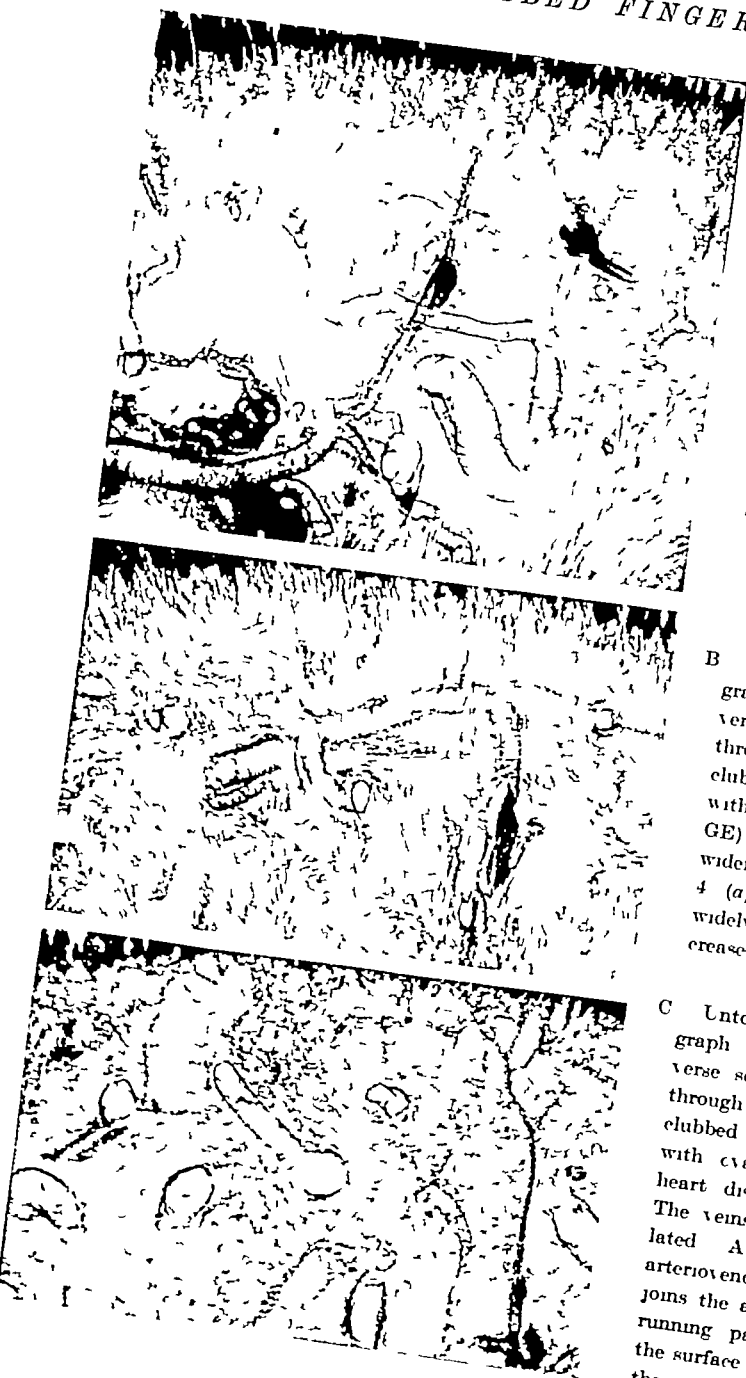
A   Untouched photomicro
graph (× 40) of a trans
verse section 200μ thick
through a normal nail bed
(Spec GB)   Note the
extensive venous plexuses
The   normal   arterial
arrangement is shown by
the course of the artery
entering low on the left
Arising from it are two
groups of darkly stained
arteriovenous anastomoses

B   Untouched photomicro
graph (× 40) of trans
verse section 200μ thick
through nail bed of a
clubbed finger associated
with bronchiectasis (Spec
GE)   The veins are little
wider than those in Fig
4 (a) but appear more
widely separated by in
creased interstitial tissue

C   Untouched photomicro
graph (× 40) of trans
verse section 200μ thick
through nail bed of a
clubbed finger associated
with cyanotic congenital
heart disease (Spec G)
The veins are widely di
lated   A simple type of
arteriovenous anastomosis
joins the artery and vein
running parallel towards
the surface in the right of
the picture

Fig 4

Fig 5

(*a*) Case 1   Right thumb (× 0)
on February 2nd, 1948

(*b*) Case 1   Right thumb (× 0)
on April 16th, 1948

Fig 6   Longitudinal hemisection (× 2) of a normal finger cleared after digital arterial injection
with neoprene   The arteries only are filled   (Female aged 71 died after cholecystectomy)
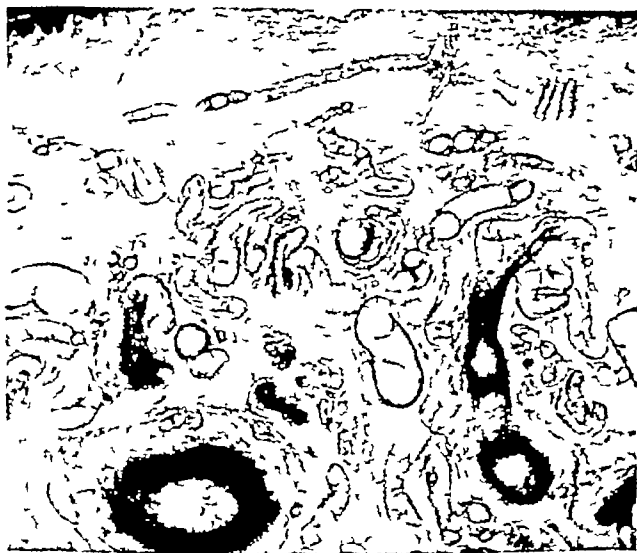
Fig 7   Untouched photomicrograph (× 40) of a transverse section 200 µ thick through proximal
part of nail bed of a normal finger (Spec GB)   Note complex venous plexus

A QUANTITATIVE STUDY OF THE RESPONSE TO COLD OF THE CIRCULATION THROUGH THE FINGERS OF NORMAL SUBJECTS

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THE response of the circulation in the fingers to local cold has been studied by Lewis (16) who used a thermoelectric junction to measure the resulting changes in skin temperature   This method provided qualitative information After 5–10 minutes immersion in water at less than 15–18°C repeated transient rises in skin temperature of a few degrees centigrade occurred, indicating periods of vasodilatation   To these the term hunting reaction was given   The temperature rises were much greater if the water was unstirred   A much larger rise in temperature, the so-called after reaction, followed the removal of the finger from its cold environment   This has recently been re-examined by Wolff and Pochin (22) using similar methods It is clear, however, as was recognised by Lewis, that during the period of immersion the observed skin temperature depends on many factors besides the blood flow through the finger   The degree of stirring of the cold water bath is particularly important   This is shown by an experiment in which the stirring was varied while the finger was immersed   (Fig 1)

The subject, a normal male, age 30, had a thermoelectric junction, A, covered by 3 layers of plaster, as used by Lewis (16), fixed to the dorsal surface of the distal phalanx of the left index finger, just proximal to the nail, and a second thermoelectric junction, B, held against the pulp of the distal phalanx by a very light rubber band   At 0 minutes the finger was immersed to the proximal interphalangeal joint in a stirred water bath at 2 5°C   The junction A cooled slowly, and B rapidly, to temperatures just above that of the water   The temperature at both junctions began to rise at 5 minutes, A reaching a considerably higher temperature than B by 9 minutes   At this point the stirrer was stopped   There was an immediate small rise at A, and a great rise at B   When the stirrer was restarted at 1 3 minutes there was a more rapid temperature fall at B than at A

The difference between A and B up to the 9th minute was mainly due to the temperature gradient through the 3 layers of plaster which lagged the junction A   While the stirrer was operating B was very nearly at the

temperature of the water bath, and presumably would have come even closer to it if the stirring had been more effective A, on the other hand, was at a temperature intermediate between that of the inside of the finger and that of the water bath

When the stirrer was stopped, A was lagged by the plaster plus still water, and B by still water only The still water clearly gave an important, though uncertain, degree of lagging, because both junctions now registered high and nearly equal temperatures
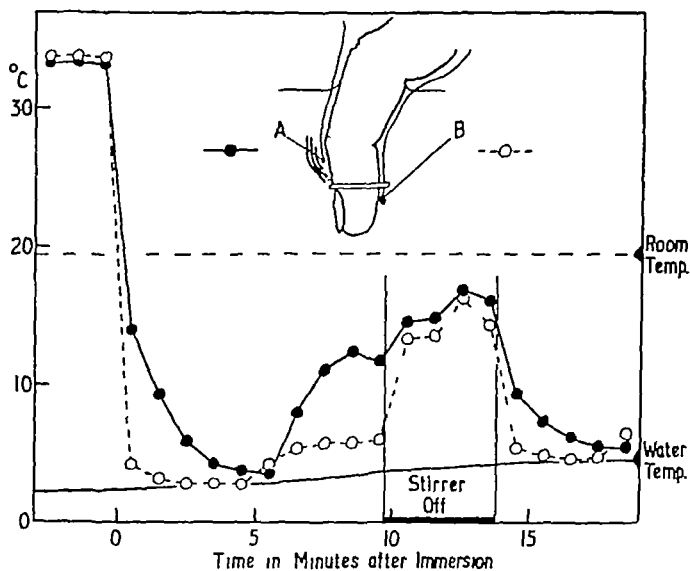

Fig 1 To show the effect of stirring a cold water bath on the temperature recorded at the surface of the finger at two points, A and B The thermoelectric junction at A was covered by 3 layers of adhesive plaster

Only rough qualitative deductions can therefore be made from temperature measurements in the unstirred water bath It can be argued that in the stirred water bath the external surface of the plaster is at the temperature of the water, and that the difference between the temperature at A and water temperature is proportional to the amount of heat passing through the plaster This quantity is probably dependent on the blood flow, but it is not possible to make a quantitative estimate of the level of blood flow from the temperature observations

It was therefore decided to make observations on the finger with the venous occlusion plethysmograph In the course of this work it became apparent that this method is unreliable during exposure to cold because of the greatly reduced capacity of the finger vessels even at the height of the cold vasodilatation Calorimetric observations were therefore made, and it is believed that these provide reliable quantitative information This paper presents both the plethysmographic and calorimetric findings

<center>Methods</center>

<center>*The temperature-controlled venous occlusion plethysmograph*</center>

This instrument has already been described (11)   The finger was inserted almost to the proximal interphalangeal joint, into a thin and loosely fitting fingerstall, which was invaginated into the copper plethysmograph   A thermoelectric junction between the skin and fingerstall recorded the temperature on the dorsum of the first phalanx   The plethysmograph was filled with water, and a head of pressure which could be altered at will, but which was normally 7 cm of water, kept the fingerstall applied to the finger
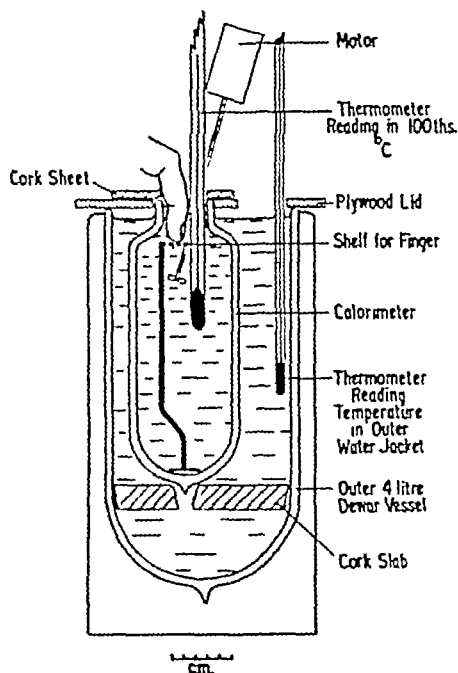
<center>Fig 2   The calorimeter for the finger</center>

The volume changes were transmitted by air to a soap bubble volume recorder (9)   The photographic record showed the pulsation size without distortion   (Fig 13)   The collecting pressure normally 70 mm Hg   was applied by a cuff at the root of the finger   The hands were arranged on a comfortable inclined surface about 10 cm above a horizontal plane through the sternal angle of the sitting subject   The copper plethysmograph was surrounded by a water bath and by changing the water temperature the temperature at the dorsum of the finger could be varied as required

The measurement of heat exchanges between the finger and its environment

Most calorimeters for the fingers have suffered from the defect of having a correction of the same order as the quantity being measured   (1, 18) As it was proposed to work with an unusually large temperature difference between the calorimeter and the surroundings, it was clearly essential to reduce these corrections   The calorimeter used to overcome these difficulties is shown in Fig 2   It was a development of the calorimeter for the hand described by Greenfield and Scarborough (10)

The calorimeter proper was a Dewar flask of 1 litre capacity   This was filled with water at the desired starting temperature   It rested on a cork slab inside a 4 litre Dewar vessel   The top of the 1 litre flask was flush with the upper surface of a plywood lid, clamped to the 4 litre vessel   The intervening space was filled with water at approximately the same temperature as the inner vessel, and this temperature did not change more than a fraction of 1°C during an experiment   The inner flask was thus provided with an environmental temperature which was nearly constant and close to that being measured   This made the temperature correction extremely small

The inner flask was provided with a small perforated shelf 3 5 cm from the top, supported from below on a light brass framework   This defined the position of the finger tip   A gap in the shelf permitted a motor driven stirrer and a thermometer* calibrated in hundredths of a degree Centigrade over an adjustable 6° range to pass more deeply into the flask The stirrer and thermometer were held by vertical rods fixed to the plywood The finger, thermometer and stirrer passed through holes in a 5 mm thick paraffin waxed cork sheet, which slid in metal guides into position over the mouth of the 1 litre flask   Similar calorimeters, but with 480 ml or 650 ml inner flasks were used for making control observations on the opposite forefinger in the 29–32°C range

Throughout the calorimetric experiments observations were made on the distal 2 8 cm of index finger   In the earlier experiments (Figs 8, 9 and 12) the volume was measured by making a plasticine mould and filling it with water to which a small amount of soap solution was added to reduce the surface tension   Repeated measurements on the same subject on the same occasion agreed within 0 3 ml   Later (Figs 10 and 11), Lennard-Jones' method (14) was used, but modified by the provision of a stop for the finger 2 8 cm below the top surface   Repeated results agreed within 0 1 ml A post 2 8 cm high was erected on the calorimeter shelf   In preparing the calorimeter the inner vessel was filled with water until, with the stirrer and thermometer in position, the water level just reached the top of the post

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* Supplied by B Black & Son Ltd , 180 Goswell Road, London

An amount of water equal to the finger volume was removed from the calorimeter so that on insertion of the forefinger the depth of immersion was 2 8 cm
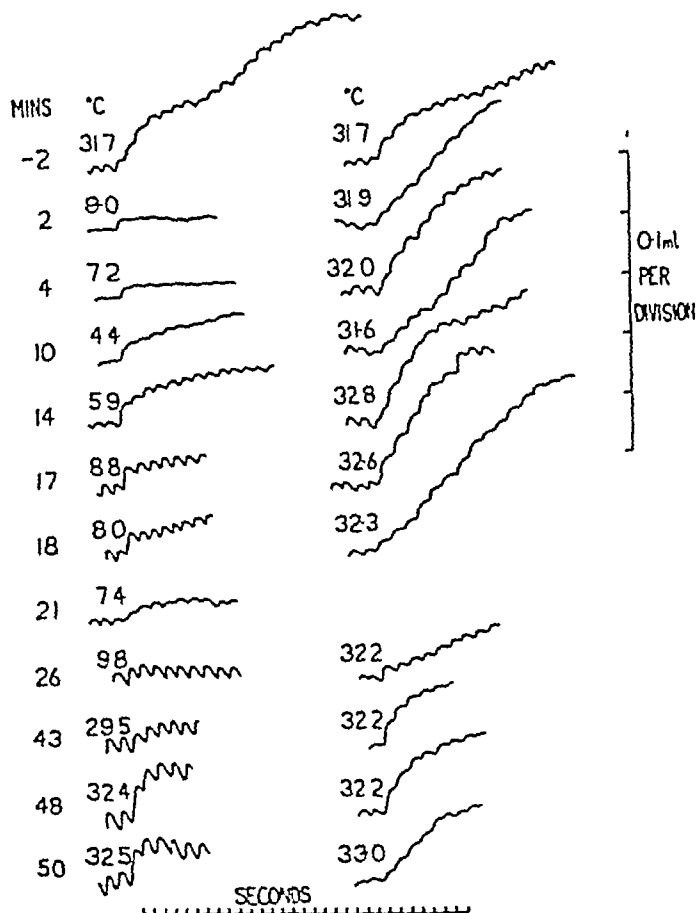

Fig 3 Plethysmographic tracings from both forefingers showing the effects of inflation of a collecting cuff at 70 mm Hg   The figures on the left show the time in minutes from the start of the cooling   The temperatures adjacent to each tracing are the temperatures at thermoelectric junctions in contact with the skin of the dorsum of the distal phalanx   From the start until 17 minutes, the pressure head on both fingers was 7 cm of water, thereafter it was 27 cm.   This increase of external pressure did not prevent blood accumulating on the control side nor did it assist the accumulation on the cold side, as it would be expected to do if the capacity vessels were distended

In using the calorimeter the consequences of immersing one forefinger were compared with those of immersing the opposite comparable finger, but with the circulation arrested   The starting temperature of the calorimeter was arranged to be as nearly as possible the same in each case and the fingers were in water baths at the same temperature, usually 29–30°C , for

at least 20 minutes before transfer to the calorimeter   Differences between the two sides were then considered to be due to the blood flow in the finger with the circulation free

RESULTS

Plethysmographic observations

The subjects for these experiments rested for a period of 45 minutes in their normal indoor clothes at a room temperature of 18–20°C before recordings commenced

A plethysmograph was placed in position on each forefinger and the temperature of the surrounding water bath was adjusted to give readings of 32°C at the thermoelectric junctions on the dorsum of both fingers This temperature was maintained on the left (control) side throughout the experiment   On the right side, after a basal period of 20 minutes the water in the outer chamber was replaced by ice to reduce the temperature at the thermoelectric junction as rapidly as possible to about 4°C and to maintain it close to this temperature for 40 minutes   At this point, the temperature was quickly raised again to 32°C for a further period of 35 minutes Plethysmographic records were made at intervals throughout   Fig 3 shows reproductions of the records from a typical experiment on one of the five normal subjects tested   The top pair of tracings show the effect of applying a pressure of 70 mm Hg to the collecting cuff on the right, and then the left index finger   The water in the outer chamber of the right plethysmograph was replaced by ice at 0 minutes and an attempt was made to keep the thermoelectric junction on this side at about 4°C until 40 minutes when the temperature at this junction was raised to 31–33°C as quickly as possible, and then maintained at this temperature

The effects of these procedures can be seen in the remaining tracings The tracings on the control side showed only changes which might be expected under resting conditions   On the cooled side, from 0–9 minutes the pulsations were nearly abolished, and no blood accumulated in the finger on application of the collecting pressure   The lack of accumulation of blood could have been due to an absence of inflow, or to a failure of the capacity vessels to provide accommodation for such blood as did enter Calorimetric evidence presented later, showed that the inflow of blood almost ceased under these conditions , it was therefore difficult to obtain information about the state of the capacity vessels   The sudden rise in the record on applying the collecting pressure was partly, and perhaps wholly, an artifact, as it was also seen if the collecting pressure was applied while a cuff at 200 mm Hg was maintained on the upper arm

After 10 minutes the pulsations became rather larger, and it was possible to collect some blood in the finger   The pulsation size continued to increase, but the volume of blood accumulating in the finger on application of the

collecting pressure at 26 minutes was actually less than at 18 minutes   In view of Lewis's observations (16), the large pulsations, and the difficulty now experienced in keeping the finger at a low temperature, it seemed likely that at this stage the lack of accumulation of blood was due to lack of accommodation rather than lack of arterial inflow, a view supported by calorimetric evidence given later   This could have been due either to constriction or to full distension of the capacity vessels   The latter explanation seemed unlikely as the finger was raised above the sternal angle, but to get further evidence on the point, the pressure head on both fingers was increased from 7 to 27 cm of water from the 17th minute onwards   It was thought that if the vessels were in fact distended this pressure might partly empty them and so allow blood to accumulate when the collecting pressure was applied   As it did not have this effect, it is probable that the failure to obtain an increase in volume was due to constriction of the capacity vessels

Warming the cooled finger increased the pulsation size slightly and restored some of the capacity, as shown by the tracing at 43 minutes   After this the pulsation size remained about the same, but the capacity of the finger vessels progressively increased and the steepness of the inflow curves implied a high blood flow

In other experiments, the external pressure head of water on the finger was increased to as much as 50 cm with similar results   making it very unlikely that the capacity vessels were so distended that they could not accept more blood   Varying the collecting pressure up to 100 mm Hg did not materially alter the amount of blood collected in the finger during cold vasodilatation, but some increase was found at 110 mm Hg although the quantity was less than on the control side at the same pressure   The capacity vessels were therefore not easily distended

On account of the greatly diminished capacity of the finger vessels during the cold period, it was impossible to draw a tangent to the inflow curve before this flattened out, and this method of measuring the blood flow could not be used   After the cooled finger had been warmed to 32°C for a few minutes, however, there was usually sufficient return of capacity for accurate tangents to be drawn to at least 3 pulse waves before any flattening out occurred

Blood flow results obtained in this way are shown in the lower half of Fig 4 and correlated with the temperature as measured on the dorsum of the fingers   The changes in blood flow on the control side were consistent with the general variations in vasomotor tone to be expected at the room temperature used   On the opposite side no blood flow measurements could be made from the records during the period of cold   When the temperature on this side was rapidly returned to 32°C the blood flow greatly exceeded

that on the control side   After 5 minutes at this temperature the flow through the right index finger was 64 ml per 100 ml per min , whereas the flow through the left (control) finger was only 15 ml per 100 ml per min

The duration of this after-reaction was not determined, but there was little diminution in flow during 35 minutes of observation   All subjects tested showed this high level of blood flow when the finger temperature was quickly changed from 4–10°C up to 32°C
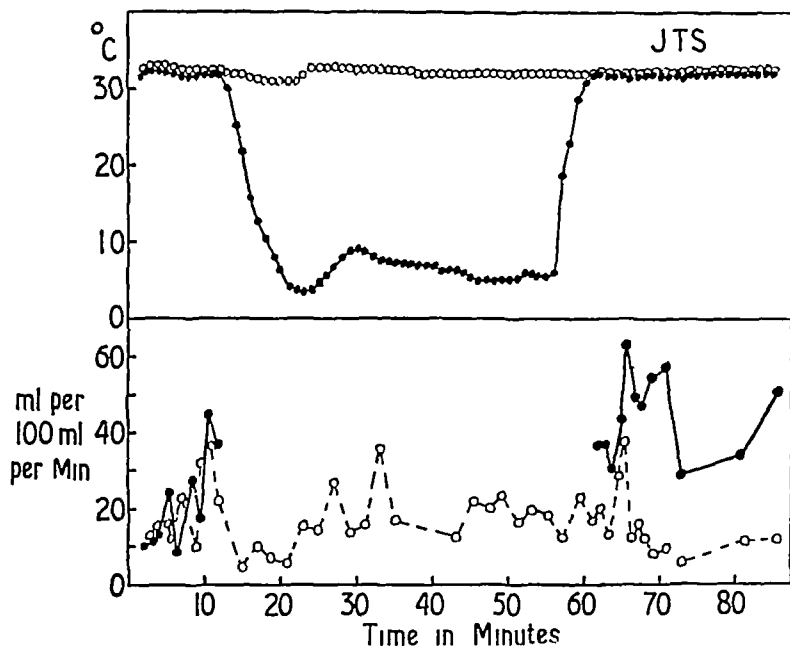

Fig 4   Blood flows in the forefingers determined plethysmographically   The temperatures of thermoelectric junctions in contact with the skin of the dorsum of the distal phalanx are shown in the upper half of the figure   Blood flow measurements are shown on the control side (open circles and dotted lines) throughout, and on the cooled side (solid circles and continuous lines) before and after cooling

Calorimetric observations

The results of a typical experiment are shown in Fig 5 and the thermometer readings in the right hand calorimeter at the start and finish of the experiment are shown in Table I

The subject was a healthy medical student age 19 years   During observations he sat on a chair, wearing indoor clothing with the sleeves down, at a room temperature of 19 5–20 8°C   Both hands were immersed in water at 29°C for 30 minutes before transfer to the calorimeters   The left (control) hand was removed from the water and dried, and one minute later the forefinger was immersed in a calorimeter at 28 98°C for 5 minutes before beginning observations   The temperature was then read at minute intervals from — 4 minutes to + 84 minutes   At + 75 minutes a cuff was inflated

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to 200 mm Hg on the left arm   Two minutes later the temperature in the calorimeter became constant, and remained so until observations ceased   It was therefore concluded that the temperature changes during the period of observation on this side were entirely due to the circulation of blood through the finger, and no heating or cooling correction was necessary
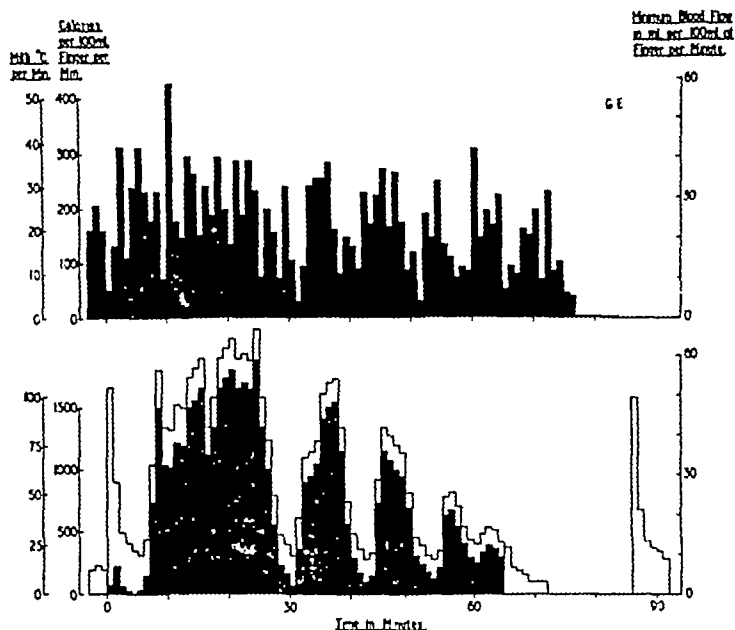

Fig 5   The temperature rise per minute recorded in the control calorimeter (left side, upper chart) at 29 98–30 88°C and in the cold calorimeter (right side lower chart) at 1 27–5 59°C as shown by the interval between the top line and the abscissa   Deductions about the heat loss from the finger due to blood flow, and the minimum level of blood flow are shown by the black polygons   On the left side the top line coincides with the black polygon The heating correction of the cold calorimeter was greater in this experiment than in any other experiment reported in this paper

The upper half of Fig 5 shows the temperature rise per minute in millidegrees C and the heat elimination in calories per 100 ml of finger per minute for the control finger

Meanwhile the temperature of the right calorimeter was read at minute intervals   At — 1 minute the right hand was removed from the water bath at 29°C and dried, and at 0 minutes the right forefinger was inserted into the calorimeter, which was at 1 270°C   At + 65 minutes a cuff was inflated to 200 mm Hg on the right arm   By + 69 minutes the temperature rise in the calorimeter had settled to a steady value of 6 milli °C per minute At + 85 minutes the left forefinger with the circulation still arrested, was removed from the left calorimeter now at 30 883°C and dried   It was inserted at + 86 minutes into the right calorimeter, which had been cooled

to 1 592°C in the interval between + 77 and + 85 minutes   Observations were continued until + 92 minutes by which time the rate of temperature rise had become nearly constant at 18 milli °C per minute

TABLE I

Subject G E   Finger volume R 5 9 ml , L 5 8 ml   Calorimeter volume R 935 ml
L 488 ml

(Observations concerned with blood flow in the right index finger only)

Time in min	Calorimeter temp C	Temp rise per min, milli °C	Temp correction per min, milli °C	Temp rise per min due to circulation of blood, milli °C	Heat eliminated per min, cals per 100 ml finger	Minimum blood flow per min, ml per 100 ml finger
Right index finger, circulation free						
0	1 270					
1	1 375	105	100	5	90	2 5
2	1 432	57	43	14	222	6 9
3	1 464	32	27	5	79	2 5
4	1 490	26	24	2	32	1
5	1 512	22	22	0	0	0
6	1 532	20	18	2	32	1
7	1 560	28	18	10	158	4 9
8	1 626	66	18	48	761	23 8
9	1 740	114	18	96	1520	47 5
10	1 825	85	18	67	1060	33 2
11	1 908	83	18	65	1025	32 1
63	5 452					
64	5 485	33	6	27	427	13 4
65	5 512	27	6	21	333	10 4
Right index finger, circulation occluded						
66	5 536	24				
67	5 550	14				
68	5 562	12				
69	5 572	10				
70	5 578	6 ⎫				
71	5 584	6 ⎬ This figure used for correction at 64–65 min				
72	5 590	6 ⎭				
Left index finger, circulation occluded						
86	1 592					
87	1 692	100 ⎫				
88	1 735	43 ⎪				
89	1 762	27 ⎪				
90	1 786	24 ⎬ These figures used for correction at 0–6 min				
91	1 808	22 ⎪				
92	1 826	18 ⎭				

Correction between 6 and 64 min on a sliding scale from 18 to 6 milli °C

The temperature rise each minute in the right hand calorimeter is shown in the lower half of Fig 5 by the distance between the upper line and the abscissa   In this case it was clearly necessary to make allowance for heat contributions to the calorimeter other than from the circulating blood   From 0 to + 6 minutes the correction applied was the temperature rise in the corresponding minute from + 86 to + 92 minutes   At + 65 minutes the correction applied was the steady temperature rise per minute observed from + 69 to + 72 minutes with the circulation occluded   Between + 6 minutes and + 65 minutes the correction was applied on a sliding scale between the two extreme values   Table I shows details of the calculations   The black area in the figure represents the temperature rise per minute and the heat elimination in calories per 100 ml of finger per minute considered to be due to the flow of blood through the finger

Blood flow through the finger

It has become clear (5) that Stewart's (19) original method of calculating the blood flow through the hand from the heat elimination, on the basis that the blood arrives at general body temperature, and leaves at calorimeter temperature, is based on false assumptions, and gives too low a value   The blood may arrive below general body temperature (2), and it may leave above calorimeter temperature (13)   The same is probably true of the finger   It seems safe to assume, however, that in the experiments now being considered, the blood could not have arrived at the finger above general body temperature, nor could it have left below the lowest calorimeter temperature   On this basis, Stewart's method may be used to arrive at a minimum figure for the blood flow   By how much the actual blood flow may have exceeded this minimum value cannot be calculated from the experimental data available, but the average internal temperature of the finger at certain stages during cooling (12) suggests that the blood may leave the finger well above calorimeter temperature

There remain, however, two uncertainties, both of which could lead to too high a value by Stewart's method, namely the metabolic heat formed in the fingers, and heat conducted down the finger from blood flowing in the non-immersed region   Heat conducted down the finger from other sources is taken into account in the heating correction previously described

If the metabolic rate of the finger is the same as that of the body as a whole it is about 3 calories per 100 ml of finger per minute   This is a generous estimate, but even so it is very small compared with the quantities of heat measured in these experiments, which may amount to over 2,000 calories per 100 ml of finger per minute during cold vasodilatation   Further, unless the metabolic rate of the finger promptly falls when the circulation is cut off our method of determining the heating correction makes allowance for it

The amount of heat conducted down the finger from blood circulating in the proximal non-immersed region was estimated by the following experiment   (Fig 6)   The right index finger was immersed in a calorimeter containing water at 0 62°C and the temperature rise each minute observed
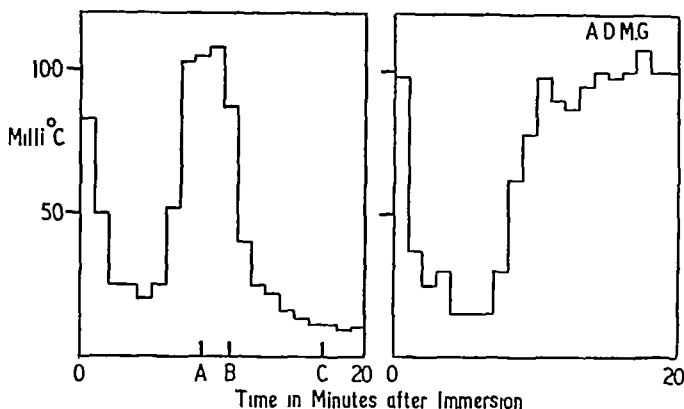

Fig 6   Results of an experiment carried out to determine the amount of heat conducted down the finger   The temperature rise per minute in milli °C during immersion of the distal 2 8 cm of the right index finger into calorimeters at 1 19°C (left) and 0 62°C (right) for 20 minutes   At A, the finger was removed for 10 seconds during which a rubber band was applied and released at the water level   (2 8 cm from the tip of the finger) At B, this procedure was repeated but the band was left in position for the remainder of the experiment to obstruct the circulation   At C a cuff was inflated to 230 mm Hg on the upper arm

for 20 minutes   This served as a control   Later in the day, the same finger, starting at a similar temperature, was again immersed in a calorimeter containing water at 1 19°C   When the cold vasodilatation had reached the plateau the finger was removed for 10 seconds, during which a tight rubber band was applied to it at the water level, immediately released, and the finger replaced in the calorimeter   This appeared to make little difference to the subsequent observations, and served as a control for the next procedure   Two minutes later, while the plateau was still maintained, the finger was again removed for 10 seconds, the rubber band reapplied tightly in the same position, and left for the remainder of the experiment to obstruct the circulation to the immersed portion of the finger only   This was followed by a rapid decrease in the rate of temperature rise in the calorimeter, which seven and a half minutes later had dropped from 108 to 11 milli °C per minute   All this time the circulation through the proximal part of the finger was free, and therefore the conduction of heat from the circulating blood down the finger to the immersed part continued   The amount of heat so conducted was estimated by arresting the circulation with a cuff on the arm at 230 mm Hg   This made practically no difference

to the temperature rise each minute   It was concluded that the quantity of heat conducted down the finger from the blood circulating in the non-immersed part was an unimportant source of error in the experiments to be described
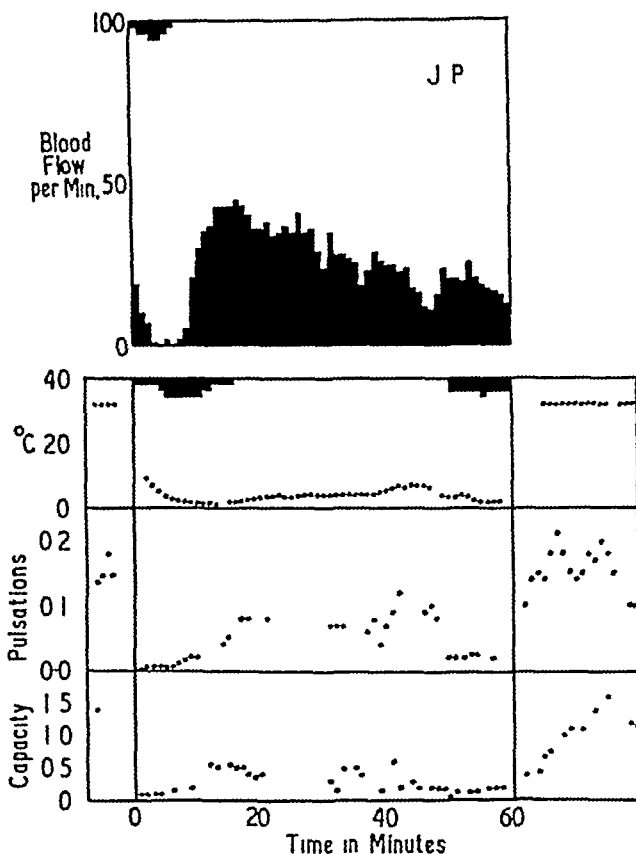

Fig 7   A simultaneous comparison during local cooling of calorimetric blood flow observations on the right index finger with the characteristics of the plethysmographic record from the opposite finger   Blood flow per min., pulsation size and capacity at 70 mm Hg collecting pressure are expressed in ml per 100 ml of finger   Pain is represented on a roughly quantitative scale by the marks below the upper lines

It appears therefore, that in spite of these uncertainties Stewart's method is essentially satisfactory for determining a minimum figure for the finger blood flow, and that this minimum may often be considerably below the actual level   Nevertheless even considering the minimum figures obtained for the blood flow the results are very striking, and the calorimetric observations reported in this paper are presented in this form This allows a fairer comparison to be made between effects at different temperatures than would be the case if results were expressed in the more usual form of heat elimination
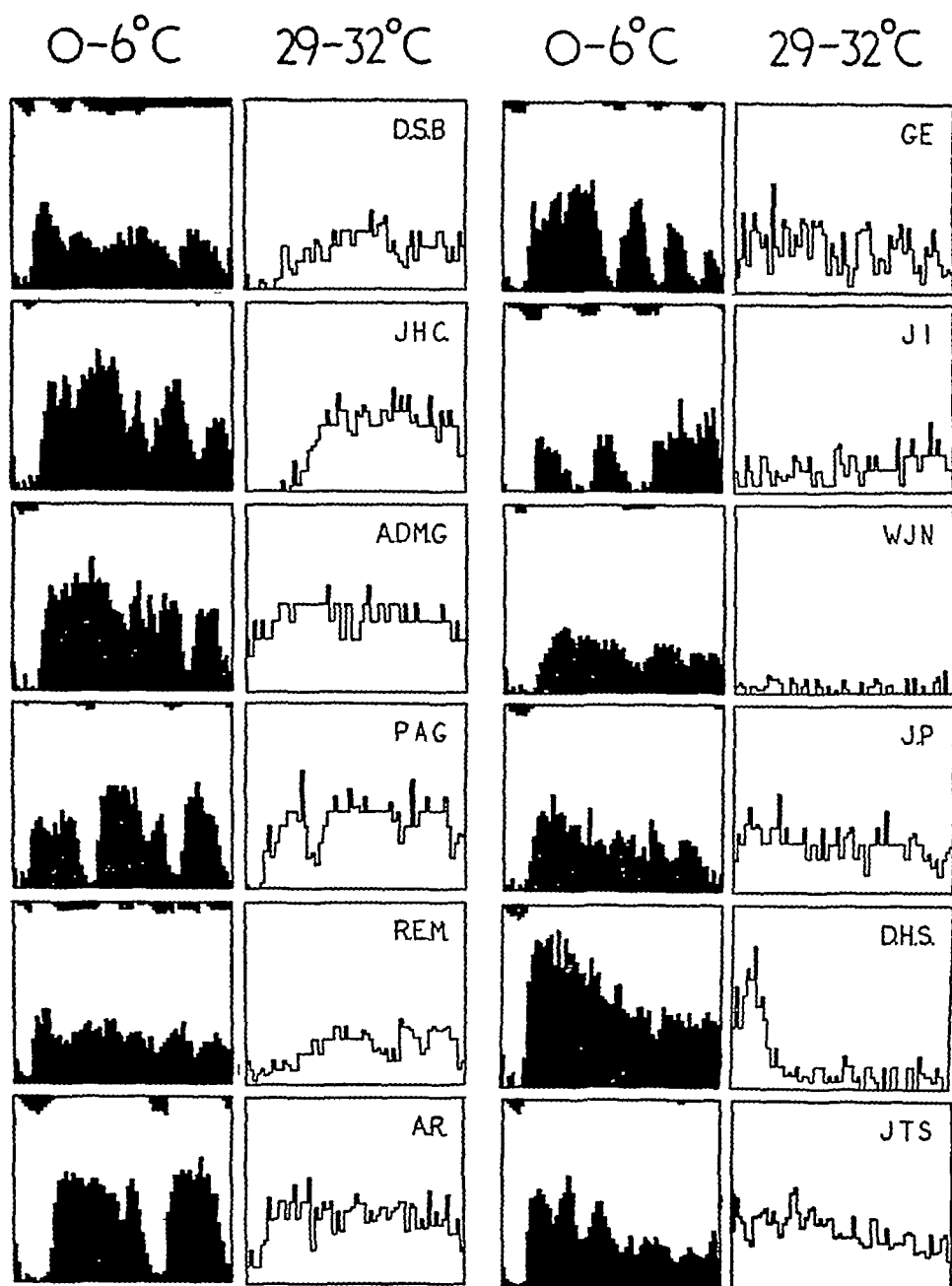
Fig 8   The minimum blood flow through the forefinger of 12 normal subjects in water at 0–6°C for 60 minutes (black figures) compared with the minimum blood flow through the opposite forefinger in water at 29–32°C (open figures)   Pain is represented on a roughly quantitative scale by the marks below the upper lines   The full height of each frame represents a blood flow of 100 ml per 100 ml of finger per minute and the full width a time of 60 minutes
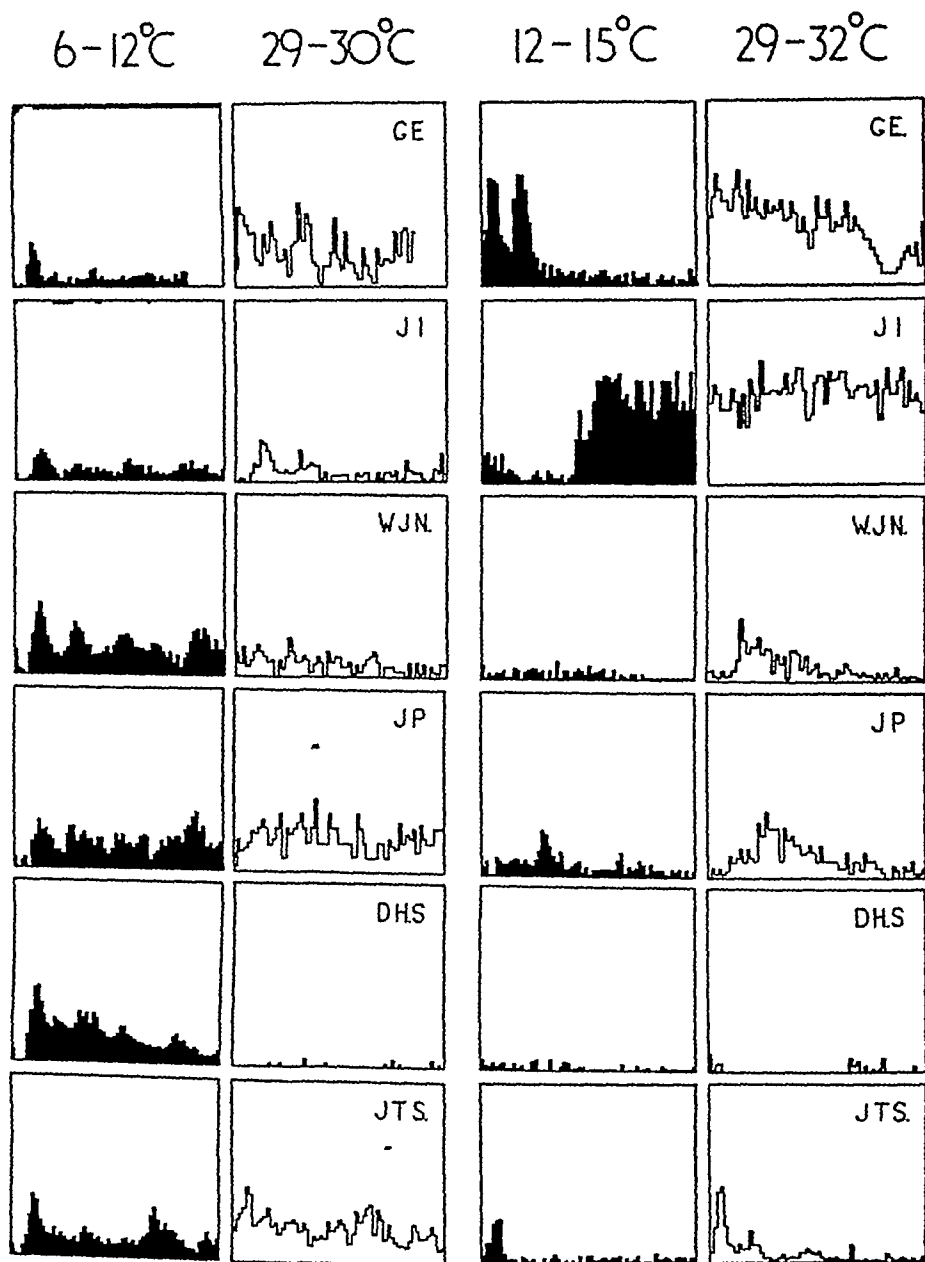
Fig 9 Observations on the minimum blood flow through the forefingers of the 6 subjects shown in the right hand column of Fig 8 repeated at 6–12°C range and 12–15°C range Other conventions are as in Fig 8

In one experiment calorimetric observations were made to determine the minimum blood flow through the distal 2 8 cm of the right index finger and correlated with simultaneous plethysmographic recordings from the distal two phalanges of the left index finger   (Fig 7)   Although no precise correlation between the blood flow on the right and the pulsation size on the left could be expected on account of the different quantities of tissue being observed and the fact that the forefingers may not behave identically, their general trend was similar   That is, during the initial period of reduced blood flow the pulsations were almost absent, and during the period of high flow they were large   On the other hand, the changes in capacity did not parallel changes in pulsation size or blood flow   For example, as compared with the initial values at 32°C , after 30 minutes cooling the capacity was reduced to about one-seventh, but the pulsation size only to three-quarters

The range of response to cold in normal individuals

Calorimetric observations in the 0–6°C range were made for 60 minutes on one forefinger of 12 normal individuals, 11 being males aged between 19 and 32, and one a female aged 47   Observations were made simultaneously on the opposite forefinger in the 29–32°C range to provide information about the general level of vasomotor tone   The room temperature varied on different occasions from 17 5°C to 22 5°C   (Fig 8) In every case the blood flow almost ceased for the first 5–10 minutes, and then increased very rapidly to between 30 and 81 ml per 100 ml of finger per minute The height attained and the subsequent behaviour showed large individual variations, the general trend in the majority being a decline from the first high value   In some cases this decline was gradual, in others periods of negligible blood flow intervened at irregular intervals

Similar observations on 6 of these 12 subjects were made in the 6–12°C and 12–15°C ranges, and the results are shown in Fig 9   At 6–12°C the blood flow almost ceased for the first 4–5 minutes, and then rapidly increased to a maximum after about 7 minutes of immersion   The majority again showed a general decline in flow for the remainder of the hour   The maximum, which varied from 17 to 42 ml per 100 ml of finger per minute, and the general level were both well below the corresponding values in the 0–6°C range   At 12–15°C there was no clear evidence of an initial intense vasoconstriction, nor of a subsequent rapid vasodilatation   The general level of blood flow was much lower throughout the hour of immersion than in either of the other two ranges   Fluctuations in blood flow on the control side, probably due to spontaneous changes in vasomotor tone, were in some cases (J T S and J P) accompanied by similar fluctuations on the cold side The striking changes in flow in the cold fingers of G E and J I , both of whom were in a state of general vasodilatation, are discussed later

Constancy of the response to cold in the same individual at similar environmental temperature

Four of the subjects used for the observation shown in Fig 8 were tested again at an interval of several days under similar conditions, but control observations on the other forefinger were omitted (Fig 10) A comparison of the two figures shows a striking similarity in the size and pattern of the responses in the same individual
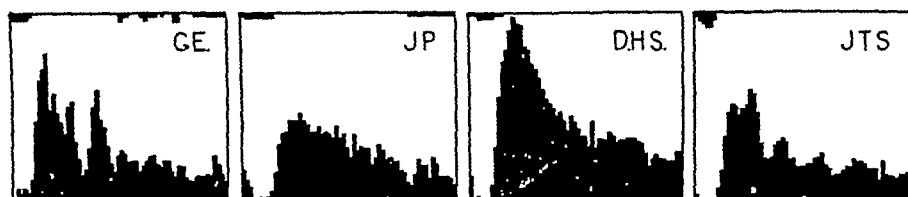

Fig 10 The minimum blood flow through the same forefinger of 4 of the subjects shown in Fig 8 retested under similar conditions Conventions as in Fig 8
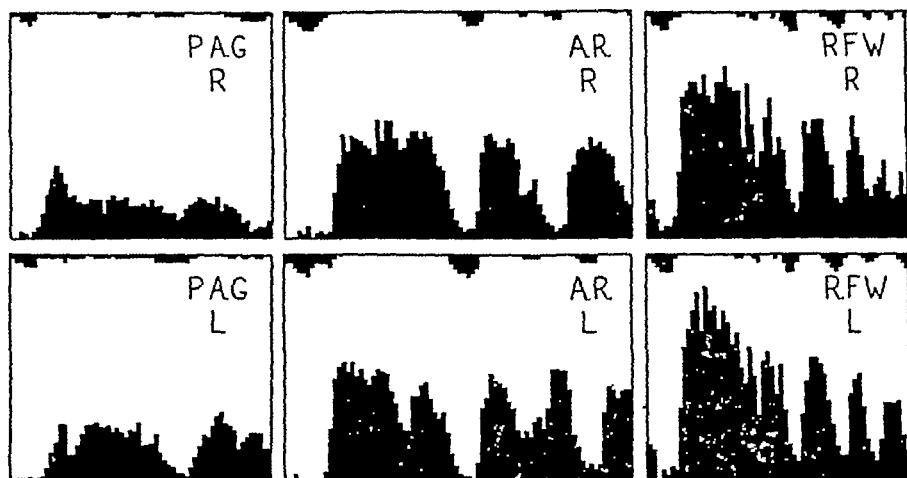
Fig 11 Simultaneous comparison of the minimum blood flow through the forefingers of 3 normal subjects during immersion in water at 0–6°C P A G and R F W., 60 minutes immersion, A R 80 minutes immersion Other conventions are as in Fig 8

Simultaneous observations of the effect of cold on the right and left forefingers

Three experiments were performed in which a simultaneous comparison was made of the minimum blood flow in the right and left forefinger while immersed in water at 0–6°C (Fig 11) The results show a striking symmetry, not only in the timing of the cold vasodilatation, but also in its size The subsequent periods of vasoconstriction and pain remained in step in P A G and R F W , bu gradually got out of step in A R These findings are similar to those of Lewis (16), who concluded that while the

oscillations occur after sympathectomy, the times at which they start are influenced to a greater or lesser extent or from time to time through the central nervous system   The fact that the oscillations may be asynchronous makes it unlikely that Kunkle (15) is correct in regarding them as gross exaggerations of the normal spontaneous and rhythmic fluctuations in blood flow (4), which like the changes in finger volume (3) are symmetrical, and are not found after sympathectomy
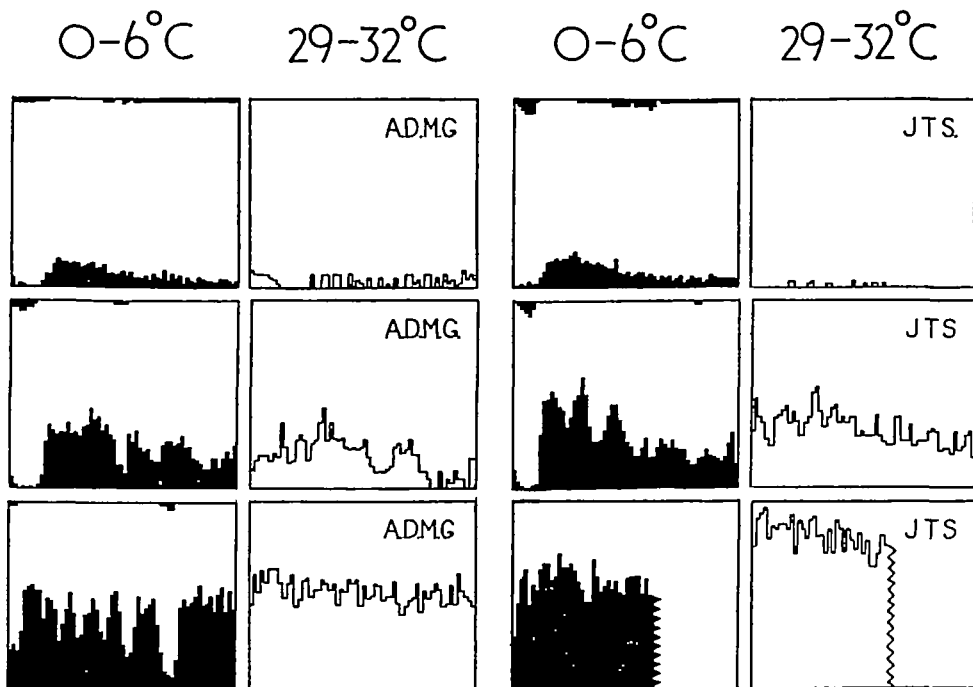

Fig 12   Observations on 2 subjects at 3 levels of peripheral blood flow, due to different general environmental conditions   The middle figure for J T S is the same experiment as is shown in Fig 8   Conventions as in Fig 8   Cold side, 0–6°C , control side 29–32°C
A D M G   *Top* figure   Room temperature 15 3–16°C   Trunk and legs bare, both arms wrapped in towels, sitting in front of two electric fans (calorimeters screened)
  Middle figure   Room temperature 14 5–13 8°C   Trunk and arms bare   No fan, but doors and windows open
  Lower figure   Room temperature 24 2–25 2°C   Subject wrapped in blankets, feet in stirred water bath at 44°C
J T S   *Top* figure   Room temperature—no record but probably 16–18°C   Trunk, legs and left arm bare, right arm covered   (Right finger in cold calorimeter)   Sitting in front of electric fan
  Middle figure   Room temperature 19 2–20 4°C   Indoor clothing, sleeves down
  Lower figure   Room temperature 24 5–25°C   Subject wrapped in blankets, feet in stirred water bath at 44°C   Experiment discontinued at 37 minutes because the limit of the thermometer scale had been reached

The effect of the general level of vasomotor tone on the response to cold

On two subjects, observations were made at 3 levels of general vasomotor tone, namely vasoconstriction, vasodilatation, and an intermediate range   These were induced by exposure of the subject to varying

environmental conditions (Fig 12) In both subjects the general level of minimum blood flow in the finger exposed to water in a calorimeter at 0-6°C was much less, and the pain more severe and prolonged, in the vasoconstricted than in the vasodilated state, the significance of these observations is discussed later When vasodilated, there was no initial period of almost absent blood flow

Pain

In a preliminary series of experiments it was noticed that pain, which was generally severe for the first 6-7 minutes after immersing the finger in water at the 0-6°C range, disappeared quite suddenly as the blood flow increased, and was usually replaced by a feeling of warmth, so that the finger in cold water often felt subjectively as warm as or warmer than the control finger in water at 29-32°C Records were therefore kept of the pain sensation, a scale from 1 (just perceptible) to 5 (unbearable) being used The occurrence of pain is shown in Figs 7-12 by marks along the top line, and the striking relationship of pain to low blood flow is quite clear When a cold vasodilatation, sufficiently great to give a sensation of warmth in the finger was followed by a vasoconstriction causing insufficient drop in blood flow to cause pain, a sensation of cold was experienced

None of our subjects was able to identify the two types of cold pain described by Kunkle (15) Between one-half and one minute after immersion at 0-6°C pain commenced, and gradually increased in severity Within a few minutes the finger became numb, so that the shelf in the calorimeter could not be felt, and this numbness to touch and pressure was often accompanied by a reduction but usually not by a loss of pain When the vasodilatation occurred, there was a rapid disappearance of pain, with no stage of "second" pain, and the touch and pressure sensations returned as the finger warmed At the same time a wave of cold or burning sensation passed up the shaft of the finger

Discussion

There is clearly a difference in the response of the capacity and peripheral resistance vessels of the finger to local cold Throughout exposure of one hour to water at 0-9°C the capacity vessels are more or less constricted The peripheral resistance vessels, after an initial period of constriction, are dilated, but the dilatation is often interrupted by short periods of constriction The exact anatomical identity of the resistance and capacity vessels is uncertain Grant, Bland and Camp (8) have shown that in the rabbit's ear local cold after an initial constriction opens up arteriovenous anastomoses and smaller arteries and veins, while the capillaries remain constricted or dilate only slightly The capillaries are capable of dilating in response to irritation As it is not known, however, what proportion of the total accommodation is provided by these various vessels when a collecting pressure is applied, it is difficult to correlate these findings with our own

The reduced capacity during exposure to cold may be due to active contraction of the capacity vessels, or to an alteration in the rigidity or viscosity of the surrounding tissues   We have no decisive evidence, but the finding of large pulsations in the plethysmograph record at the time of reduced capacity makes it improbable that increased tissue rigidity or viscosity plays an important part

Whether the cold dilatation is equal to the maximum obtainable is not certain   The calorimetric method can only supply with certainty a minimum value for the blood flow, but the evidence available suggests that the dilatation may be maximal   While it is unlikely that the quantity of cold blood returning from the single immersed finger tip pre-cools the arriving arterial blood to more than a fraction of the degree found when the whole hand is cooled (2), some precooling is likely   If the venous blood leaving the finger is at the average internal finger temperature, which we have found to be 20–30°C at the height of cold vasodilatation (12) the actual blood flow is not less than twice the values shown in this paper   In fact the venous blood might leave the finger at a higher or lower temperature than the average for the whole finger , it depends on the anatomical relationship of the vessels to the various isothermal surfaces in the finger, and other considerations such as local heat interchange between arteries and veins   We have no information about the latter, but during cold vasodilatation in the rabbit's ear Grant (6) observed that the bulk of the blood passed through the arteriovenous anastomoses, and Grant and Bland (7) have produced evidence that the same is true of the finger   In the finger the anastomoses are situated in the deeper parts, at the level of or slightly superficial to the sweat glands (7), a depth at which the temperature is probably not far removed from the average for the whole finger   The maximum blood flow through the distal two phalanges was found by Wilkins, Doupe and Newman (20), using a finger plethysmograph, to vary from 60 to 120 ml per minute per 100 ml of finger, figures which agree with Burton's findings (4), and from the information in their paper it can be calculated that the blood flow through the distal phalanx is from 90–180 ml per minute per 100 ml of finger   Mendlowitz (18) observed 11 normal subjects during indirect heating and found a maximum heat elimination from the finger tip into a calorimeter at 30 4 to 31 6°C of 361–619 cals per 100 ml of finger per minute Calculating the corresponding minimum level of blood flow in the way used for our own results, a range of 66 to 115 ml per 100 ml per minute is arrived at   If it is correct to double our minimum figures for cold vasodilatation to obtain the true figures, the flow at the height of dilatation in our experiments at 0–6°C was 60–192 ml per minute per 100 ml of finger, which suggests that dilatation was complete

The question arises to what extent the local effects of cold and the central vasomotor control of the blood vessels can support or oppose each other   Lewis (16) has shown that while the cold reaction can still be

obtained after sympathectomy, and after section but not after degeneration of the mixed nerves, it is more or less influenced by general vasomotor activity. It seems that at 0–6°C a slight cold vasodilatation can occur in the face of general vasoconstriction. Examples of this are shown in Figs 8 and 12. In interpretating these results it must be remembered that equal precooling of the arterial blood arriving at the finger would lead to a greater proportionate reduction in the heat elimination into a calorimeter at 29–32°C than into one at 0–6°C, and this gives rise to some uncertainty as to whether, in the vasoconstricted state, the true level of blood flow is really greater on the cold than on the control side. This uncertainty is much less in the experiments in Fig 8 than in those in Fig 12 because the latter were conducted with the subject largely naked in a cold room with fans operating. Lewis (17) on the basis of skin temperature observations concluded that cold vasodilatation may fail if the body is chilly, or if the tone of the more proximal vessels of the limb is increased by exposure to cold. Our own evidence, while not conclusive, suggests that some degree of cold vasodilatation may still occur in these circumstances.

The calorimetric method gives a much higher minimum value for the blood flow in the cold finger at the height of cold vasodilatation in warm than in cold subjects (Fig 12). An exact comparison cannot be made between the results on the observational fingers, because although we kept the observational arm clothed in each experiment we are not certain that the arterial blood always arrived at the finger at the same temperature. It is unlikely, however, that this uncertainty accounts for all the difference noted, and we conclude that the size of the cold vasodilatation is greater in the warm than in the cold subject.

The most striking effect of a release of vasomotor tone is the absence of the initial vasoconstriction at 0–6°C (Fig 12), which is regularly found in subjects exposed to comfortable room temperatures (Fig 8). This is presumably because the internal temperature of the finger is kept high by the flood of warm blood.

At 6–12°C and especially at 12–15°C the vasodilatation in response to cold is much smaller than at 0–6°C and it is less well sustained. After a few minutes the blood flow falls to a low value, and the flow on the cold side may be well below that on the control side. In the 12–15°C range if the subject is warm there may be a sudden release from this cold vasoconstriction and once this release has taken place the circulation may keep the finger too warm for the cold to reassert itself (Fig 9, J I). On the other hand the local vasoconstrictor effect of cold may assert itself after a considerable interval (Fig 9, G E). Under these conditions the finger blood vessels are in an unstable condition, and either local or general influences may temporarily predominate.

No arterial pressure measurements were made in the experiments reported in this paper, largely because the blood flow to the fingers would have been disturbed   We feel, however, that the observations on the control finger show that arterial pressure changes could not have accounted for the changes in flow recorded on the cold side   We are uncertain to what extent changes in blood viscosity in the cold finger may have been responsible for changes in blood flow which we have interpreted as changes in vessel calibre   We know of no viscosity observations using a limb viscosimeter which are relevant to the conditions in our experiments

The findings on pain can be explained on the following simple hypothesis An internal finger temperature close to 0°C causes pain   If this temperature is continued, touch and pressure sensations are lost, and later pain sensation also   Immersion of a finger in water at 0–6°C without arrest of the circulation, however, leads to vasodilatation and reheating of the finger, and removal of the pain stimulus   This normally happens long before the local cold can lead to a loss of pain, whether by adaptation of the nerve endings or by blocking of the nerve fibres   Later periods of vasoconstriction lead to the finger becoming cold again, frequently with a return of pain We cannot accept the suggestion of Wolf and Hardy (21) that the thermal gradient in the tissues may be the stimulus for cold pain   The gradient is clearly greatest when the blood flow is high, and when the internal finger temperature is high (12), but we have never experienced cold pain under these conditions   On the other hand, when the internal finger temperature is low, and the thermal gradient is therefore small, pain was, in our experiments, invariably experienced   It is specially noteworthy that in the two experiments in Fig 11, in which the subjects were warm, the blood flow through the cold finger was at a particularly high level and there was no initial period of vasoconstriction, no pain was experienced by J T S and only very slight pain by A D M G   In these experiments the thermal gradient in the finger was probably steeper than in any others in our series

Neither do we think it likely that the cold pain causes the vaso-constriction, but rather that vasoconstriction by lowering the internal finger temperature leads to pain   In many of our records it can be seen that the blood flow was already declining before pain was experienced, and there were many declines of blood flow which were unaccompanied by pain, though they often caused a sensation of cold

SUMMARY

1   The circulation through the finger during and after local cooling has been studied with a venous occlusion plethysmograph

2   Cooling causes a constriction of the capacity vessels in the finger, which invalidates, and makes misleading, the plethysmographic method On raising the temperature of the cooled finger to 32°C the capacity vessels relax and the method becomes valid

3 To obtain information about the circulation during cooling quantitative estimations of heat elimination from the terminal 2 8 cm of finger have been made These have been used to assign minimum values to the blood flow

4 An improved calorimeter for the finger, which enabled these observations to be made is described

5 Under comfortable environmental conditions, immersion of the finger in a water bath between 0° and 6°C (23 observations on 14 subjects) caused an initial almost complete cessation of blood flow, followed after 5–10 minutes by a rapid increase to between 30 and 98 ml per 100 ml per minute The subsequent behaviour during 1 hour of immersion varied between individuals, most showing a general decline usually with intermittent periods of greatly diminished flow

6 Similar but smaller changes in blood flow followed the initial constriction period in fingers immersed between 6°C and 12°C

7 At 12°–15°C there was no initial constriction, and the flows were generally lower than in either of the two preceding ranges

8 The response in an individual was reproducible under similar conditions, and the general form of the response of the two forefingers was the same

9 The size of the reaction appears to be influenced by the general level of vasomotor tone

10 The relationship of the minimum values to the true level of blood flow is discussed, and it is suggested that a maximal dilatation can be produced by local cooling

11 Pain was felt in the finger at 0–6°C when the blood flow was sufficiently small It was never felt during a large cold vasodilatation

12 The relationship of cold pain to the internal finger temperature is discussed and it is concluded that pain is felt when the temperature is low, and the temperature gradient between the inside and skin of the finger is small Pain is not felt when the internal temperature is high, and the gradient steep

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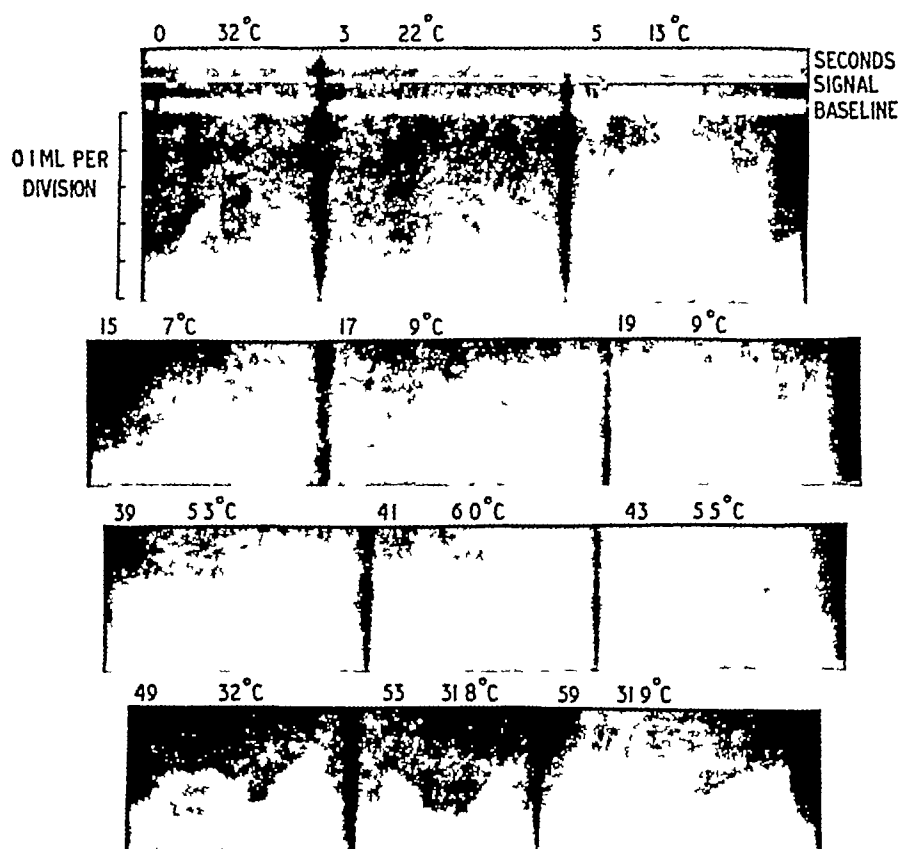(22) WOLFF AND POCHIN   Clinical Science, 1949, **8**, 145

Fig 13 Plethysmographic tracings from the right and left index finger from a typical experiment (subject J T S) The tracings are in pairs that from the cooled side being made first in each case The numbers refer to the time in minutes from the commencement of cooling and the temperatures are those at a thermoelectric junction on the dorsum of the cooled finger The control finger was at 31 5–32 5 C throughout

THE AVERAGE INTERNAL TEMPERATURE OF FINGERS IMMERSED IN COLD WATER

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A CALORIMETRIC method for the study of the heat elimination from the finger immersed in cold water has been described by Greenfield and Shepherd (1) On the assumption that the blood could not arrive at the finger above body temperature or leave below calorimeter temperature, a minimum figure can be calculated for the blood flow corresponding to any particular calorie output As it would be very difficult for anatomical reasons to measure the temperature of the mixed venous blood leaving the finger tip in order to arrive at a more realistic value for the blood flow, it was thought that a measurement of the average internal temperature of the finger might be used to give some indication of the probable temperature of the departing venous blood A knowledge of the internal temperature of the finger is also of importance in the interpretation of the fluctuations in cold-pain (1, 2)

METHOD

The principle of the method was as follows With the distal 2 8 cm of forefinger immersed in the calorimeter, temperature observations were made every minute, as described by Greenfield and Shepherd (1) The circulation was suddenly arrested Measurement of the heat subsequently transferred to the calorimeter from the finger enabled the average temperature of the finger at the moment of arrest of the circulation to be determined The method was calibrated by transferring fingers with the circulation arrested from water baths at varying known temperatures to the calorimeter, and measuring the heat released

RESULTS

The calibration of the method

Nineteen experiments were performed on 12 subjects A typical experiment will be described in detail The subject sat with his left forefinger immersed to a depth of 2 8 cm in a stirred water bath at 21 375°C

for 3 mmutes   Cuffs were then mflated at the root of the finger and on the upper arm to a pressure of 250 mm Hg   Two cuffs were employed to mcrease the probabihty that the circulation through the finger was arrested   The finger was left m position m the water bath for a further 7 mmutes which is a sufficient mterval for 98 per cent of complete thermal equilibrium to be attamed, as can be seen from Table I   It was then transferred to a calorimeter at 0 582°C , the transfer takmg less than 10 seconds   The temperature observations m the calorimeter are shown m Table I, and the

In each case the ordinate represents the obser\ed temperature rise each mmute m milli °C , the water equi\alent of the calorimeter and contents was 1020 g , and the hatched area is the part of the temperature rise each mmute due to the warmmg correction
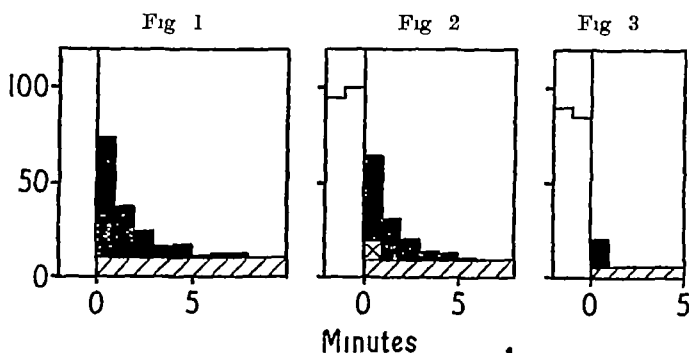

Fig 1   Fig 2   Fig 3

Minutes

Fig 1   At 0 mm the left forefinger, volume 6 6 ml , and with the circulation arrested, was transferred to the calorimeter from a water bath at 21 37°C with which it had come mto equilibrium   The final temperature m the calorimeter was 0 80°C   The black area represents the heat released from the finger m fallmg from 21 37°C to 0 80°C

Fig 2   The finger, volume 4 7 ml , and with the circulation free, had reached a stable le\el of heat elimmation at the height of cold \asodilatation, the calorimeter temperature bemg 3 68°C   At 0 mm the circulation was arrested   The double hatched area represents heat released before the arrest of the circulation, but affectmg the thermometer after the arrest The black area represents heat released from the finger as it cools to calorimeter temperature

Fig 3   The finger, \olume 4 7 ml , and with the circulation free, was at the height of cold vasodilatation   At 0 mm it was withdrawn from the calorimeter the temperature bemg 3 10°C   The black area représents the part of the temperature rise obser\ed after remo\al of the finger due to heat released from the finger before removal

results are shown graphically m Fig 1   By the 8th mmute the temperature rise each mmute had settled to a steady value of 10 milli °C   This, the heatmg correction (*see* later), was subtracted from the temperature rise m each of the precedmg mmutes to obtam the temperature rise due to the immersion of the finger

The results of a number of experiments of this type are shown m Fig 4 The number of calories released per ml of finger are plotted agamst the temperature difference between the water with which the finger was m equilibrium before transfer, and the contents of the calorimeter at the attamment of equilibrium after transfer   Had the distal 2 8 cm of finger been severed from the body, these experiments would have provided data

for measuring the specific heat   Owing to the uncertainties of heat transfer down the finger from the non-immersed region, they did not provide reliable information about specific heat, but they did provide a reliable calibration for the measurement of average internal finger temperature

TABLE I

Finger volume 6 6 ml   Water equivalent of calorimeter and contents 1020 g   Finger transferred to the calorimeter from a water bath at 21 37°C at 0 min

Time in mins	Calorimeter temperature °C	Temperature rise per min in milli °C	Heating correction per min in milli °C	Temperature rise per min due to finger, in milli °C
0	0 582			
		73	−10	63
1	0 655			
		37	−10	27
2	0 692			
		24	−10	14
3	0 716			
		16	−10	6
4	0 732			
		17	−10	7
5	0 749			
		11	−10	1
6	0 760			
		12	−10	2
7	0 772			
		12	−10	2
8	0 784			
		10	−10	0
9	0 791			
		10	−10	0
10	0 801			
				Total 122

Total heat release from the finger $= 0 122 \times 1020$ calories

Total heat release per ml of finger $= \dfrac{0 122 \times 1020}{6 6}$ calories

$= 18 8$ calories

Temperature change undergone by finger $= 21 37-0 80°C$

$= 20 57°C$

The release of heat from the finger following arrest of the circulation

In these experiments the subject inserted the distal 2 8 cm of one forefinger into water in a calorimeter at 0–4°C and the cold vasodilatation was awaited   When the rate of heat elimination from the finger had reached a steady value, the circulation was arrested by inflating cuffs at the base of the finger and on the upper arm to 250 mm Hg   In different experiments this arrest was made at different levels of heat elimination   The temperature readings from a typical experiment are shown in Fig 2

The heating correction

It can be seen in Fig 2 that the temperature rise each minute approached a value of 9 milli °C , and became constant at this value from the 7th minute This was taken to be the heating correction, and was subtracted from the observed temperature rise during each of the preceding minutes, to give the temperature rise due to the finger

The correction for thermal lag in the calorimeter and thermometer

In any real calorimeter heat released at one point takes a finite time to become distributed through the system, and to cause a change in thermometer reading   This became a matter of importance in the present experiments, because the thermometer readings after the arrest of the circulation must have been partly dependent on heat released from the finger before the arrest of the circulation   No corresponding lag occurred in the calibrating experiments, so it was clearly essential to determine the size of this error, and to apply a correction if possible   The following experiment was therefore performed

The subject kept his forefinger in a calorimeter at 3 10°C until the cold vasodilatation had reached a maximum   He then withdrew the finger, but left the hand in position above the calorimeter   The hole for the finger was covered with a cork sheet   The temperature readings are shown in Fig 3   It can be seen that the heat released from the immersed finger at the height of the cold vasodilatation became distributed throughout the calorimetric system within one minute of its withdrawal, and that even in this minute the temperature rise only amounted to an extra 13 milli °C This is small compared with the quantities being measured   The precise correction presumably depends on the rate of heat elimination from the finger in the minute before the arrest of the circulation, and this varied in different experiments   It was thought, however, that a reasonable correction would be to subtract, in all cases, 10 milli °C from the temperature rise in the first minute following arrest of the circulation

The relationship of internal finger temperature to blood flow

Seventeen observations were made on 12 subjects and the minimum figure for the blood flow through the finger was calculated for the minute preceding the arrest of the circulation in each case (1)   The relationship between this figure and the average internal temperature of the finger is shown in Fig 5   It can be seen that at the height of cold vasodilatation in a finger immersed in water at 0–4°C the average internal temperature was often between 20°C and 30°C   Since the superficial parts of the finger are at a temperature considerably less than this, some of the inner parts must be at a temperature considerably higher

DISCUSSION

The overall errors of the method are those of the calibration procedure, plus the error due to thermal lag   The last has been shown to be small   The errors in the calibration procedure can be seen from Fig 4 to be proportionately smaller at high finger temperatures than at low ones   The evidence in Fig 4 makes it unlikely that the temperatures in Fig 5 are in error by more than ± 4°C at any point on the scale
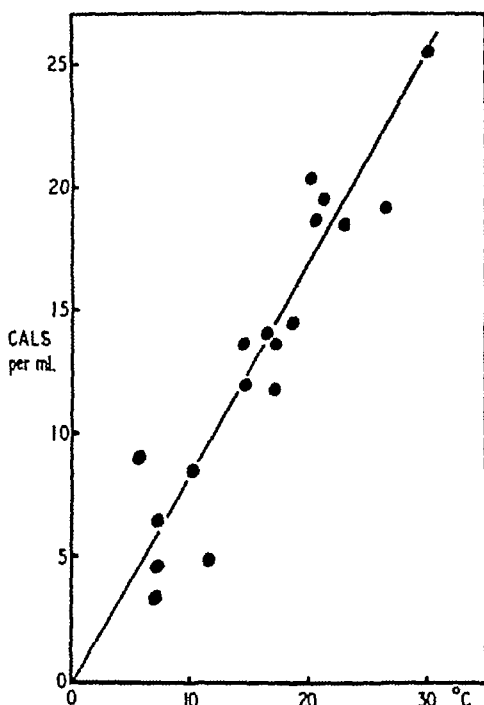

Fig 4   The release of heat in calories per ml of finger (ordinate) when the finger undergoes various temperature falls (abscissa) on being transferred from a water bath at various temperatures to a calorimeter at 0–4°C   The circulation was arrested in every case   The straight line was used in subsequent experiments (Fig 5) to convert observed calorie release to temperature change   It corresponds to an apparent specific heat of 0 75

The information we obtain in this way has certain advantages over that obtainable with thermoelectric junctions   Firstly, the structure of the finger is not disturbed   In a relatively small organ like the finger tip, the trauma caused by inserting a thermoelectric junction may modify the circulation to an indeterminate degree   Secondly, the overall average temperature is obtained in place of the local temperature in a very small region

Measurements with a thermoelectric junction at the naked surface of the finger (1) show that in a stirred water bath or calorimeter the temperature there is substantially that of the water   It can therefore be deduced that

under these conditions the temperature gradient through the tissues of the finger is proportional to the difference between the water temperature and the average internal finger temperature   The implications of such deductions in relation to the mechanism of cold-pain are reported separately (1)
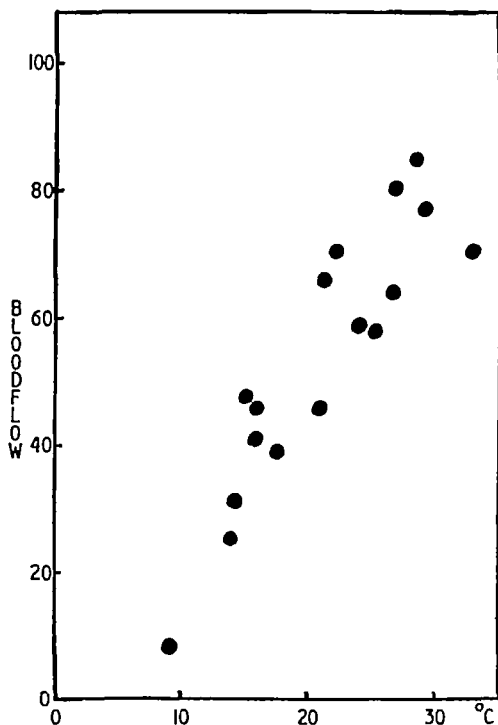

Fig 5   The average internal temperature of the forefinger (abscissa) at varying stages of cold vasodilatation shown (ordinate) in terms of the minimum value of the blood flow in ml per 100 ml of finger per min

SUMMARY

1   A calorimetric method is described for determining the average internal temperature of the finger

2   The relationship of this temperature to the blood flow through the finger during the vascular reaction to local cold has been investigated

3   At the height of cold vasodilatation due to immersion of the distal phalanx in a stirred calorimeter at 0–4°C the average internal temperature of the finger is 20–30°C

4   The temperature gradient in the finger varies with the difference between the average internal finger temperature and the water temperature

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THE EFFECT OF ACUTE OCCLUSION OF THE FEMORAL ARTERY ON THE BLOOD SUPPLY TO THE CALF OF THE LEG BEFORE AND AFTER RELEASE OF SYMPATHETIC VASOMOTOR TONE

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FOLLOWING occlusion of the common femoral artery the limb is dependent for its blood supply on the arterial anastomoses around the hip In the present investigation the blood supply to the calf of the leg has been studied in young healthy subjects following acute occlusion of the femoral artery Observations have been made under resting conditions and after exercise before and during indirect heating and before and after injection of tetraethylammonium bromide (T E A B)

Methods

The femoral artery as it passes under the inguinal ligament, rests partly on the ilio-pectineal eminence and partly on the tendon of psoas muscle, which itself is supported by bone , these structures therefore provide a firm base against which compression can be maintained A mechanical compressor was used to occlude the artery This consisted of a hinged horizontal arm fastened at one end to a table , a wooden block 5 cm by 3 5 cm surmounted by a 1 5 cm thick rubber pad was attached to the under surface of the free end of this arm, and this pad was centred over the artery The weight necessary to occlude the artery was determined by perfusion experiments in the cadaver, and it was found that a 9 5 kg weight, placed directly over the wooden block, was more than sufficient to prevent flow, even with perfusion pressures up to 160 mm Hg This weight could be borne by most subjects for a period of 8-10 minutes without discomfort

Blood flows were recorded through the calf of the leg by means of venous occlusion plethysmography before, during and after application of compression (3) As the arterial pressure in the limb following occlusion of the femoral artery was unknown, the most suitable collecting pressure was estimated by recording calf flows at different pressures, the one giving the

highest apparent arterial inflow being chosen   The collecting pressures ranged from 20-75 mm Hg   During observations a cuff on the ankle was inflated to 200 mm Hg to arrest the circulation to the foot (7)

Indirect heating was carried out by immersing in a stirred water bath at 44 5°C , the hand, forearm, and as much of the arm as possible on the side opposite to that on which the compression was applied, and heating was further encouraged by wrapping the subject in blankets

T E A B was administered as a 10% solution (100 mg per ml) intravenously and 500 mg , which is the maximum dose advised, was the amount injected in all cases

The use of body heating as a means of releasing sympathetic vasomotor tone is already recognised (2, 4, 5, 6, 8)   This procedure, as well as releasing vasomotor tone in the normal circulation, should act in a similar manner on the collateral circulation if this is also governed to any extent by the sympathetic nervous system   T E A B acts by blocking transmission at the autonomic ganglia, both sympathetic and parasympathetic (1) If this drug, therefore, by blocking transmission through sympathetic ganglia, will increase the normal limb blood flow, it should also increase the calibre of the collateral vessels if these are tonically constricted by the sympathetic nervous system

RESULTS

The calf blood flow under resting conditions after femoral occlusion

Resting calf blood flows were recorded and when these became stable the compression was applied to the femoral artery and the calf blood flow again determined   An initial drop in flow to about one-sixth of the resting value occurred on arterial occlusion and this was followed by a rapid recovery, the blood flow returning to approximately its previous resting level within 1-6 minutes, average time 2 minutes   Pulsations were absent from the plethysmographic record for times varying from 1 to 8 minutes from the onset of compression, the average time being $2\frac{3}{4}$ minutes   Even at the end of 10 minutes, pulsations were usually slight and in no case was their amplitude comparable with those present in the normal circulation   No pulse could be detected in the posterior tibial or the dorsalis pedis artery during the occlusion

The body was then warmed to release sympathetic tone and when calf flows showed no further increase the femoral artery was occluded and flows again measured   Occlusion produced a great fall in blood flow   The flow soon increased, and in the more or less stable period between the 2nd and 4th minutes, the flows during indirect heating averaged 2 4 times those obtained in the same subject at the same time after arterial occlusion but

without indirect heating in the 12 cases tested (Fig 1) In 2 of the 12 cases no increase was demonstrable even though the heating had increased the flows before occlusion from 1 9 to 7 3 ml and from 1 5 to 5 6 ml /100 ml calf/min respectively

A second similar series of tests was then carried out in which 500 mg of T E A B was injected intravenously to release vasomotor tone   The femoral artery was occluded at times varying from ¼ to 3½ minutes after the injection   In one case the injection was commenced simultaneously with
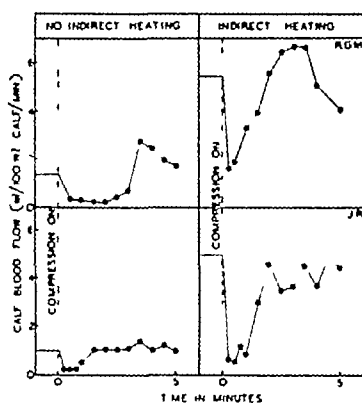

Fig 1   The blood flow through the calf of the leg before and during occlusion of the femoral artery of an unheated subject, compared with similar observations during indirect heating The dotted line indicates the application of compression   The flow to the left of this line is the average calf flow through the normal circulation
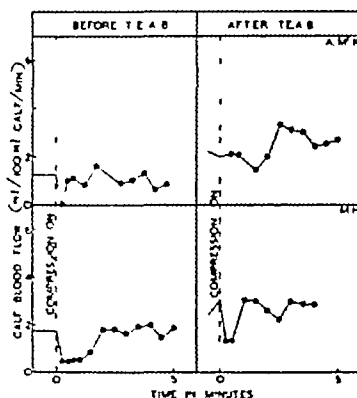

Fig 2   The blood flow through the calf of the leg with femoral occlusion before and after T E A B intravenously

the arterial occlusion   Fig 2 shows typical results   Nine subjects were tested   In all after arterial occlusion the flow at the peak of the response to T E A B was increased on the average 1 5 to 2 times, over that in the same subject at the same time after occlusion without T E A B

The calf blood flow following exercise after femoral occlusion

With the compressor in situ and the calf enclosed in a heavy water-filled brass plethysmograph, the only practical method of exercise consists of dorsi or planter-flexion of the ankle   As the posterior calf muscles form a much larger group than the anterior it was decided to use these muscles in carrying out the exercise   For these experiments an assistant stood facing the subject's feet, and with his hands cupped against the ball of the foot to be tested and his elbows placed against his own body, he dorsiflexed the subject's foot to its fullest extent   The subject, with his shoulders supported to prevent his slipping up the couch, attempted to planter-flex his foot to his maximum capacity against this resistance, the assistant keeping the foot in the dorsiflexed position by exerting counter pressure   In fact, the exercise carried out was a repeated approximately maximum isometric contraction of the posterior calf muscles   This exercise was carried out 5 times in 30 seconds, 5 seconds continual contraction and one second relaxation between contractions   Immediately after the last contraction the subject relaxed and a flow was recorded   It was found possible to record this first flow 3 seconds after interrupting exercise and this time interval was, therefore,

TABLE I

Subject	Average of 3 highest calf flows after exercise (ml /100 ml calf/min)		Calf flows with femoral occlusion expressed as percentage of calf flows without femoral occlusion
	Without femoral artery occluded	With femoral artery occluded	
C N	32 2	0 4	1
D A E	32 5	3 1	10
G T H	33 8	3 3	10
R G S M	30 3	5 2	17
H McC	33 0	6 2	19
J G	38 6	8 0	21
W T B	30 7	10 7	35
T H	27 5	12 0	44

used throughout these exercise experiments   As soon as this flow had been recorded another exactly similar series of contractions was made, the blood flow recorded after 3 seconds, and the process repeated until the subject wanted to stop

Exercise was carried out in 12 subjects in whom the calf flow had been measured after occlusion of the femoral artery at rest   Eight of these

exercised with and without femoral occlusion, 4 only with femoral occlusion Four exercised without occlusion before repeating it with occlusion , the other 4 reversed the order

Exercise and calf flow measurements were continued for times varying from 3¾ to 10 minutes, average 6 minutes, after occluding the femoral artery An aching pain in the limb forced the subjects to stop in all but two instances Table I compares the averages of the three highest flows for each subject without femoral occlusion with the averages of the three highest flows with femoral occlusion, and in the last column the latter is expressed as a percentage of the former Under these circumstances the values obtained for the collateral circulation ranged between 1% and 44% of the normal

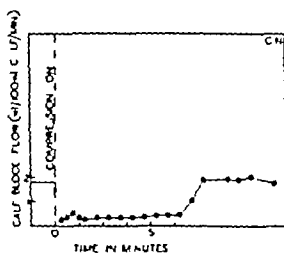Fig 3   This illustrates the longest period of decreased flow which occurred after compression of the femoral artery

There was one anomaly in these results   In the case of C N the average of the three highest flows after occlusion in response to exercise was only 0 4 ml /100 ml calf/min yet the flows after occlusion with this subject at rest were in the region of 2 ml , which was similar to the resting flow through the normal circulation   Under resting conditions this subject of all those tested had the longest period of decreased flow (5¼-6 minutes) after compression, before the normal resting level was regained (Fig 3)   He was forced to discontinue exercise after 3¾ minutes owing to intense pain in the leg and ankle

In the 4 subjects in whom the response of the normal circulation was not determined, the averages of the three highest flows after occlusion were W J A , 4 3, J T S , 5 2, M H , 10 2, J L R , 14 7 ml /100 ml calf/min As a basis for comparison the average of the figures shown in the first column of Table I was computed and found to be 32 3 ml and from this it will be seen that the collateral circulation in these 4 instances also fell well below the average level of the normal circulation   Typical results are shown in Fig 4   The mechanism by which the circulation after occlusion increased in response to exercise above the resting level after occlusion is not entirely clear   The increase in general blood pressure resulting from this exercise was probably partly responsible   In 10 of these subjects blood pressure readings were taken by auscultation immediately after each series of

contractions, corresponding as far as possible to the times at which blood flows were recorded   The maximum reading for each subject obtained from these observations is shown in Table II, where it is compared with the resting blood pressure   From the blood flow ratio shown in the last column of this Table however, it would seem that the rise in blood pressure cannot be entirely responsible   This ratio was obtained by averaging the three highest flows in response to exercise after occlusion and dividing this figure in each case by the average of the three highest resting flows after occlusion   While it is possible that some of the lower ratios could be explained by the blood pressure increase alone this could certainly not be the case in the higher ratio group where the increase in pressure was insufficient to account for the increase in flow obtained
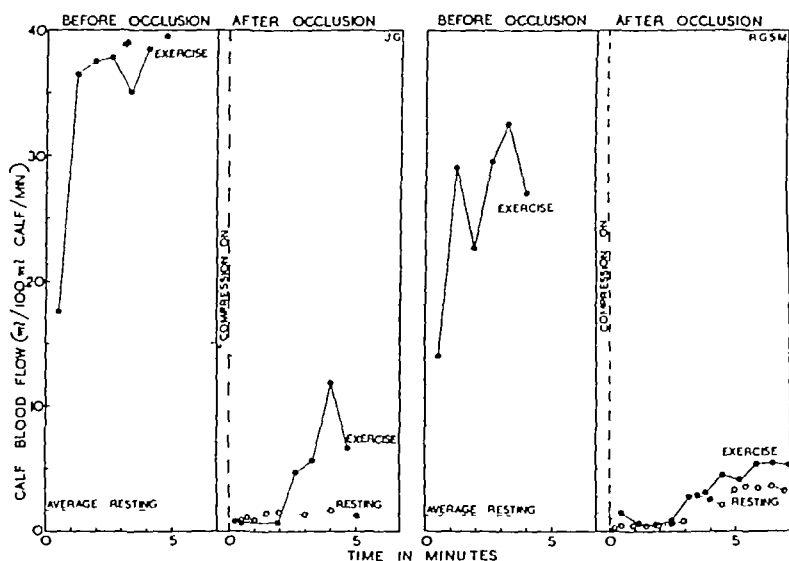

Fig 4   The response of the calf circulation to severe exercise of the posterior calf muscles before and after femoral occlusion

The effect of release of sympathetic vasomotor tone on the blood supply to the calf following exercise after femoral occlusion

To carry out this investigation, indirect heating and T E A B were again used to relax vasomotor tone and the exercise performed was similar to that just described

The results of the experiments where indirect heating was used are shown in Table III where a comparison is made between the average before and during indirect heating of the three highest calf flows after interrupted exercise with femoral occlusion   The resultant percentage increase in calf

blood flow is shown in the last column   Fig 5 shows some typical results
It can be seen that, after femoral occlusion, indirect heating caused an
increase in the maximum flow through the calf, this increase varying from
27% to 800% of the previous estimation

TABLE II

Subject	Resting blood pressure	Maximum blood pressure after exercise	Blood flow ratio
C N	110/55	120/70	1 0
W I A	134/80	144/80	1 5
R G S M	100/60	120/60	1 6
G T H	118/74	135/75	2 3
J T S	105/65	130/75	3 0
M M c C	120/80	140/80	3 4
M H	100/60	115/60	4 3
J G	100/65	120/90	5 3
T H	120/80	130/80	7 5
J J R	115/65	150/60	11 3

TABLE III

Subject	Average of 3 highest flows after exercise with femoral artery occluded (ml /100 ml calf/min)		Percentage increase due to indirect heating
	Without indirect heating	With indirect heating	
J L R	14 7	18 7	27
M H	10 2	13 8	35
T H	12 0	17 0	42
J G	8 0	11 6	45
R C S M	5 2	8 9	71
G T H	3 3	11·0	233
J T S	5 2	21 5	313
C N	0 4	3 6	800

The results obtained with T E A B are shown in Table IV where they are compared with the increase due to indirect heating It will be noted that all subjects showed an increase in flow as a result of the injection, this increase varying from 9% to 304% and also, when compared with indirect heating, there is a certain similarity in the extent of this response Fig 6 shows the response obtained in two of the cases
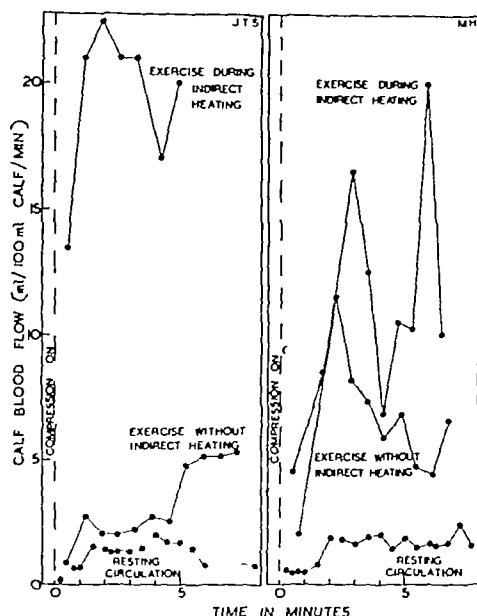

Fig 5 The effect of indirect heating on the calf circulation after femoral occlusion following severe exercise of the posterior calf muscles

TABLE IV

Subject	Average of 3 highest flows after exercise with femoral artery occluded (ml /100 ml calf/min)		Percentage increase due to T E A B	Percentage increase due to indirect heating
	Without T E.A.B	With T E A.B		
M H	10 2	11 1	9	35
T H.	12 0	18 5	54	42
H.McC	6 2	11 3	82	—
R G S M	5 2	12 6	142	71
G T.H	3 3	12 0	264	233
J T S	5 2	21 0	304	313

DISCUSSION

The results obtained from these experiments indicate that indirect heating and T E A B increase the calf blood flow in the 5 to 10 minute period following acute occlusion of the femoral artery both under resting conditions and in response to exercise   The increase in the resting flow is not due to a rise in general blood pressure resulting from these procedures since if indirect heating raised the blood pressure at all it usually did so only slightly, and T E A B generally caused no change or a slight drop in pressure   Further, there was no significant difference in the blood pressure when exercise was carried out alone, or in combination with indirect heating or T E A B
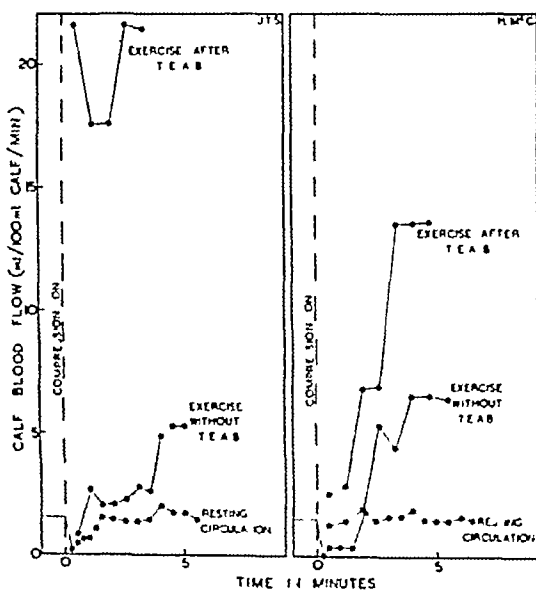

Fig 6   The effect of T E.A B on the calf circulation after femoral occlusion following severe exercise of the posterior calf muscles

Two possibilities remain to explain the increase in calf blood flow after occlusion produced by T E A B and indirect heating   The blood flow through the calf as ordinarily measured is presumably chiefly determined by the calibre of the vessels of the calf enclosed in the plethysmograph since the proximal vessels are large   After occluding the femoral artery, the proximal vessels are much smaller, and calf flow recorded under these circumstances may conceivably be affected as much by the calibre of the proximal collateral vessels as by that of the calf vessels   The increase in blood flow that occurs with indirect heating and T E A B in the presence of femoral occlusion might be due to an affect of these agents on either of the two series of vessels

mentioned   The following facts suggest that the effect of indirect heating and T E A B is largely on the collateral vessels   In a given subject exercise as described with free circulation may raise the calf flow to 35 ml /100 ml /min   Exercise with femoral occlusion produced calf flows up to 4 ml Exercise with femoral occlusion plus indirect heating or T E A B produced flows up to 14 5 ml and 13 5 ml /100 ml /min respectively   It may be assumed that exercise had produced a maximal dilatation of the calf vessels , the further increase effected by indirect heating and T E A B must be attributed to their action on the collateral vessels

Owing to the close proximity of the femoral vein and femoral artery, the method of compression used in these experiments probably occluded not only the artery but also the vein   As the extent of this occlusion was probably similar for each subject on each occasion tested, any change incurred by simultaneous compression of the vein should be relatively constant   Moreover, it seems unlikely that the venous occlusion can have affected the flows during the early period of compression, which alone is considered in this paper

It was considered whether increased formation of metabolites might occur owing to the rise in body temperature due to indirect heating and so cause dilatation of the collateral vessels   The degree of heating used in these experiments raised the body temperature about 2°F and it seems unlikely that this could cause sufficient extra metabolite formation to account for the increase in collateral blood flow above the level present without heating   In addition, T E A B , which caused a similar increase in flow, is not known to increase metabolite accumulation but owes its results entirely to blocking autonomic ganglia

It could perhaps be argued that this extra increase in flow which occurred when exercise was carried out during indirect heating or following T E A B might have been due to the previous occlusion having facilitated the re-opening of the collateral anastomoses   That this was not so was demonstrated by repeating maximum exercise on some of these subjects without indirect heating, when no extra increase was demonstrable

Finally, as indirect heating acts reflexly in releasing vasomotor tone, the efferent pathway being the sympathetic nervous system, and as the action of T E A B is due to the blocking of nerve impulses through autonomic ganglia and, what is more important in the present discussion, to the blocking of these impulses through the sympathetic ganglia, it follows that these collateral vessels are under the control of the sympathetic nervous system It is, therefore, the release of the vasomotor tone imposed by this system which permits the increase in collateral blood flow resulting from these tests

SUMMARY

1   The blood supply to the calf has been studied in young healthy adults, following acute occlusion of the femoral artery

2 Indirect heating and tetraethylammonium bromide increase the blood supply, both under resting conditions, and in response to exercise

3 This increase is due to a dilatation of the collateral vessels

4 As indirect heating and tetraethylammonium bromide act by releasing sympathetic vasomotor tone, it is the release of this control which causes the collateral vessels to dilate and the blood flow through them to increase

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SODIUM AND POTASSIUM EXCRETION IN CHRONIC RENAL FAILURE

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Introduction

It has been known for many years that salt deficiency may occur in cases of chronic renal failure (7) More recently it has been shown that in rare instances patients may develop symptoms simulating Addison's disease (10), while others die suddenly from the effects of potassium retention (3) What is really much more remarkable is that patients whose functioning renal substance as estimated by modern clearance methods, must be no more than a fifth or a tenth of the normal and whose blood urea level is greatly elevated, may yet live for months and in some instances for years, maintaining plasma levels of sodium and potassium which are strictly within normal limits

This investigation attempts to throw light upon the mechanism by which the failing kidney maintains the normal quantity of these electrolytes in the body fluids

1 Sodium

The sodium load presented to the tubules, in other words the amount of sodium filtered by the glomerulus in unit time, is represented by the product of the plasma sodium and the glomerular filtration rate In the present investigation glomerular filtration rate was estimated by the endogenous creatinine clearance, which was compared with the inulin clearance in a few instances

Thus if the plasma sodium is expressed in milliequivalents per litre, the sodium load equals $P_{(Na)} \times \dfrac{UV}{P}$ (Cr) in mEq/min where P = plasma concentration U = urinary concentration and V = urinary volume in ml per minute The question in which we are interested is how the tubules deal with the sodium load presented to them, that is, what proportion they reabsorb, and what proportion they excrete, for this is an indication of the mode of function of individual tubules Normally more than 99% of the filtered sodium is reabsorbed

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If the percentage of the sodium load which is excreted equals Y, then

the amount of sodium excreted $= UV_{(Na)} = \dfrac{Y}{100}\left(P_{(Na)} \times \dfrac{UV}{P}(Cr) \right)$,

therefore $Y = \dfrac{UV}{P}(Na) \times \dfrac{P}{UV}(Cr) \times 100$,

which is equivalent to $100 \times \dfrac{\text{sodium clearance}}{\text{glomerular filtration rate}}$

and as V is the same in each case therefore

%Na excreted $= Y = 100 \times \dfrac{U}{P}(Na) \times \dfrac{P}{U}(Cr)$

This has the great advantage of eliminating V, the biggest source of error in all investigations of this kind since it is dependent upon accurate bladder emptying and urine collection

We define renal insufficiency as a state in which as a result of chronic progressive disease, the kidney has a significantly reduced urea or creatinine clearance and is unable to produce a concentrated urine   By renal failure we mean a more advanced state in which permanent changes in the chemical structure of the body fluids (as shown for instance by a raised plasma urea or creatinine) have taken place   By this definition nearly all our cases had renal failure   The commonest causes of this syndrome are chronic atrophic pyelonephritis (with or without hypertension) ,   chronic Type 1 nephritis in the latent or terminal stage , and malignant hypertension   The pathology of our cases is shown in Table I   We have not been concerned with the œdematous syndromes of renal disease except in a few instances of terminal Type 2 nephritis, and in cases accompanied by heart failure, both of which will be referred to separately

Methods

Plasma (or serum) and urinary values for sodium, potassium and creatinine were estimated in out-patients on samples of blood and urine collected at approximately the same time   In in-patients, timed collections of urine were made (not by catheter) usually over 3 hour or 24 hour periods, to estimate creatinine clearance

The simplicity of these methods made the investigation readily applicable to a large number of out-patients who were in a state of equilibrium for the time being in the sense that their condition was not rapidly deteriorating   It was particularly these cases which we wished to study

We do not propose to enter into the controversy as to whether endogenous creatinine clearance represents a true measure of glomerular filtration rate Almost certainly it is merely an approximation   Brod and Sirota (2) have shown that it is reasonably close to inulin clearance both in normal subjects and in patients with renal failure   Black and Stanbury (1) have studied

changes in glomerular filtration rate produced by variations in blood pressure, using inulin and creatinine clearances, and have found no instance in which the change of one was not reflected by a similar change in the other although the two values were not identical   Studies on cases of severe renal failure shown in Table II of this paper show the similarity (though not the identity) of inulin and creatinine clearances and of data calculated therefrom

Creatinine was estimated by the method of Brod and Sirota (2)   Sodium and potassium were estimated by flame photometry   Inulin clearances were estimated after the subcutaneous injection of 50 ml of 10% inulin with procaine   If the first clearance period is commenced not less than an hour after the injection, stable plasma inulin levels can be maintained for several hours   Urine samples for inulin clearances were all obtained by catheter

TABLE I

Nature of disease in cases studied

	With severe hypertension	Without severe hypertension
Chronic atrophic pyelonephritis	3	6
Chronic (Type 1) nephritis	4	
Terminal (Type 2) nephritis	1	
Malignant hypertension	4	
Other renal failures (Hyperalcaemia 1 Unknown 2 Calculus, etc, 2)	2	3
Benign hypertension with renal insufficiency	3	
	17	9

Results

The normal value of Y, that is the percentage of the sodium load excreted, was worked out on healthy persons   Except on a diet abnormally low in sodium such as the rice diet, Y varies from about 0 4 to 1 5%   The ingestion of five grams of NaCl at breakfast, after a previously normal salt intake gave rise to a maximum excretion of 1 75% of the sodium load, and this was at a time of day when sodium excretion is normally at its height This may therefore be taken as the upper limit of Y on ordinary diets, and values do not usually vary more than from 0 5 to 1 5%   Fig 1 shows the results of 46 determinations of the percentage of sodium excreted in 26 patients with renal insufficiency   All but four of these had renal failure with serum creatinine levels ranging from 2 1 to 19 6 mg   Four had a lesser degree of renal insufficiency   The percentage of sodium excreted is charted against serum creatinine as an indication of the degree of renal failure   It would have been preferable to chart it against creatinine clearance but this

would have excluded a large number of out-patient observations in which accurate timing of urine collection could not be carried out and so the creatinine clearance could not be calculated   The chart clearly shows that as renal failure advances there is a general tendency for the percentage of sodium excreted to increase   It is also clear that most of the cases in which heart failure co-existed with renal failure (marked with open circles) were exceptional in showing a percentage excretion of sodium which was not commensurate with the degree of renal failure
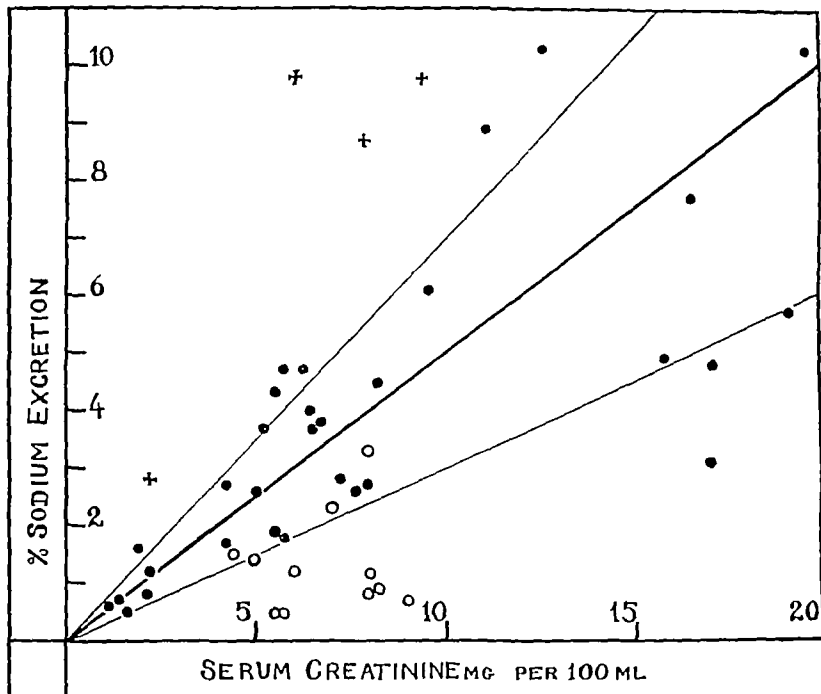

Fig 1

Discussion

Some patients with renal failure, especially cases of chronic atrophic pyelonephritis without hypertension, will live for several years with blood urea levels of more than 100 mg per 100 c c, and urea clearances of less than 10% of the average normal   It is convenient to refer to such patients as being in a state of equilibrium in the sense that their serum levels for urea and creatinine, although greatly raised above normal, do not change very much from month to month, and their serum levels for sodium and potassium are normal   In order to maintain this state of equilibrium on an ordinary diet it is clear that such patients must excrete normal quantities of urea and creatinine in a 24 hour period   This they do partly by maintaining excretion by night as well as by day, and partly by maintaining a

high plasma level of creatinine and urea so that the glomerular filtrate, although greatly reduced in volume, contains a much higher concentration of these substances Similarly patients of this kind must be excreting an adequate quantity (depending upon their diet) of sodium and potassium in the 24 hours, but this is done without any rise in the plasma level by reducing tubular reabsorption, or, in other words, by increasing the value of Y, the percentage of the sodium load excreted Referring to the original formula for the percentage of sodium load excreted

$$Y = \frac{UV}{P}\,(Na) \times \frac{P}{UV}\,(Cr) \times 100$$

since the value of UV for sodium and for creatinine will remain more or less constant, as will also the value for plasma sodium, Y must increase roughly in proportion to P (Cr), the plasma level of creatinine We have already seen (Fig 1) that this is what appears to happen When heart failure supervenes this relationship is disturbed and sodium is retained

Statistical Analysis of Data (Fig 1) It appears that there may be a linear relationship between the percentage of sodium excreted and the serum creatinine or

$$\frac{U}{P}\,(Na) \times \frac{P}{U}\,(Cr) \times 100 \; \alpha \; P\,(Cr)$$

Since P (Cr) appears on both sides of this expression it is not permissible to find if there is a correlation between them, but when P (Cr) is eliminated

$$\frac{U}{P}\,(Na) \times \frac{100}{U}\,(Cr) \; \alpha \; 1 \text{ or } = \text{a constant, F}$$

If the values of F form a series about a mean ($\overline{1}$) and do not show too much scatter it can be taken that there is a linear relationship and this can be expressed by the formula

$$\frac{U}{P}\,(Na) \times \frac{P}{U}\,(Cr) \times 100 = \overline{F} \times P\,(Cr)$$

Taking the 31 examples in Fig 1 plotted as closed circles $\overline{F} = 0\,502$ (standard error 0·035b) Although the scatter is considerable, the standard deviation being ± 0 2, the t test shows that the probability that the mean is not significant is less than 0 001

Thus the mean value of Y (% Na excreted) = 0 5 × (P (Cr)) in mg per 100 ml This relationship is shown by the heavy line, the standard deviation being shown by the lighter lines in Fig 1

Eleven cases with clinical evidence of heart failure are shown in Fig 1 by open circles $\overline{F}$ here is 0 20 (standard error 0·039) Comparison of this with the mean of the previous series shows that the difference (0 3) is significant the standard deviation of the differences being 0 105 and t = 2 87 Other cases with low salt excretion and low values of F (not included in Fig 1) were two normal subjects on the rice diet (F = 0 07 and 0 08) a case of renal failure on the rice diet (F = 0 02), and 7 cases of Type 2 nephritis with oedema ($\overline{F}$ = 0 25) Some of the latter were however, on low salt diets

A high sodium excretion is shown by the 4 cases indicated by crosses in Fig 1 F was in each case greater than 1 0 a value also seen in one normal after taking 5 gm NaCl The cases have been arbitrarily excluded from the main series It would seem that they were not in sodium equilibrium (3 of them had clinical evidence of dehydration) but examination of the figures shows that they had very low values for urinary creatinine so that their creatinine clearance may not have been representative of their G.F.R

Given that the percentage of the sodium load excreted increases as renal failure progresses the question arises as to whether this increase is fortuitous and simply due to the inability of tubules to reabsorb sodium efficiently or whether the maintenance of serum sodium is still under the influence of a controlling mechanism Failure to reabsorb the normal

percentage of sodium in the tubule could be due either to inefficient function of damaged tubule cells, or to tubular dilatation, a condition commonly present in these cases, which might lead to a state of affairs in which the axial stream had insufficient contact with the tubule cells   The rate of passage of glomerular filtrate through the tubules may also be a factor, but the fact that these cases can remain in a state of equilibrium, with a normal serum sodium, for many months at a time, seems difficult to reconcile with a purely fortuitous relationship between failure of the tubules to reabsorb and reduction in the amount of glomerular filtration
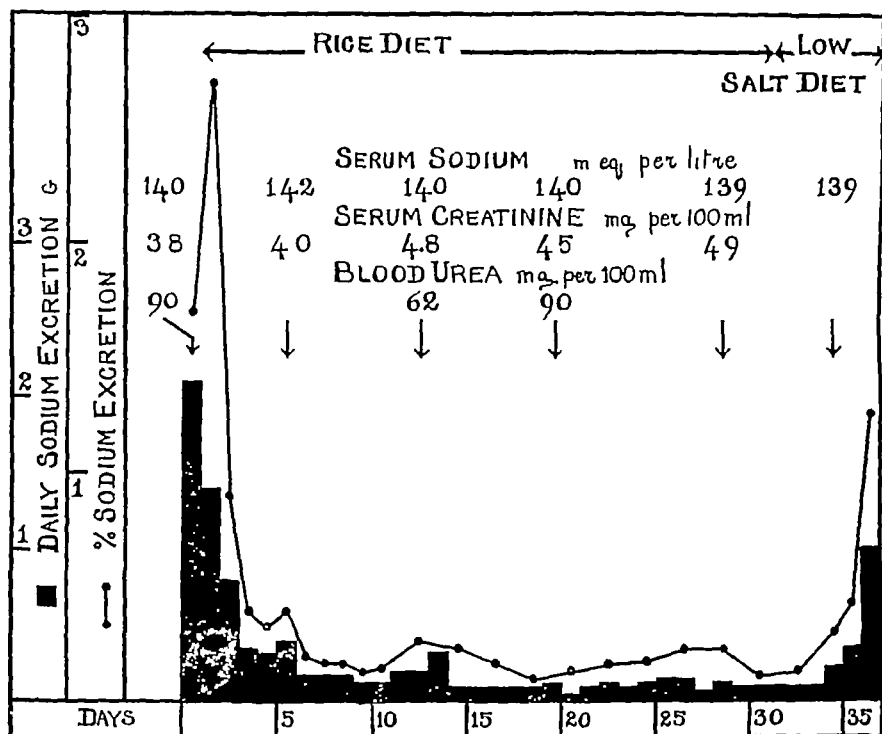

Fig 2

Most probably both factors are at work for it would seem that at least in the cases which develop salt-deficiency, the control has failed   Thorn, Koepf and Clinton (10) showed that in their " salt-losing " cases there was no response to D O C A and we have confirmed this in two salt deficient cases, but this may mean that in these exceptional instances the adrenal cortex is already exerting a maximal stimulus   It is not very easy to examine the effect of D O C A in milder cases which are in a state of equilibrium, as there are always small diurnal variations in salt excretion even when the diet is intended to be constant in sodium content   Two cases in which we have given D O C A seem to have shown a slight response

It is also difficult to judge the effect of adding extra salt   There is usually no immediate renal response to the ingestion of moderate doses (2 or 3 grams) of NaCl in renal failure, but one must not forget that many cases probably have some degree of dehydration, and that water and salt may be retained together to make up a slightly depleted plasma volume
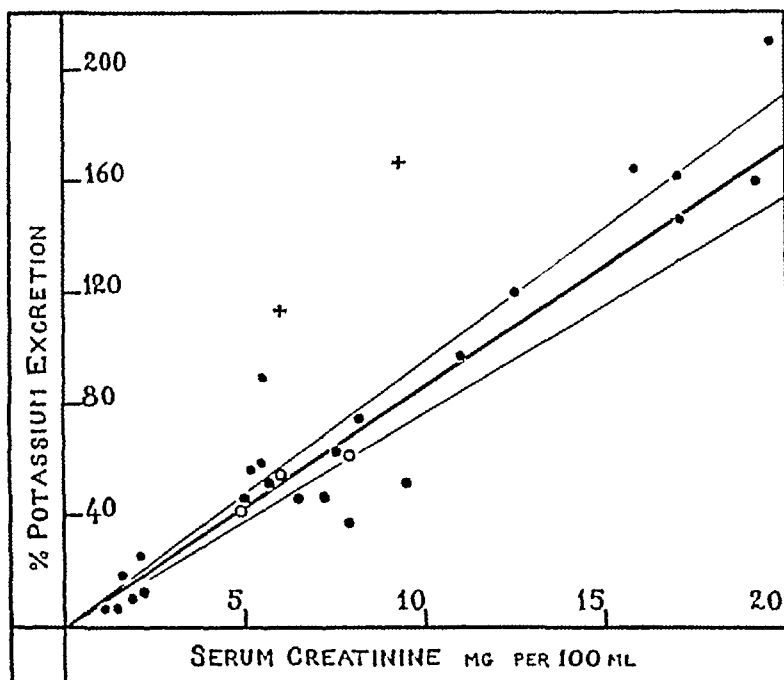

Fig 3

Two experiences seem to show clearly that in moderately advanced renal failure the tubules can still conserve sodium under certain circumstances Thus when the rice diet which is low in sodium, was taken, the percentage of the sodium load excreted, which had previously been raised, became very low and remained thus until a more normal diet was resumed (Fig 2)   The other experience is in the development of heart failure   The patient from whose data Table III was compiled was given 5 grm NaCl daily   This precipitated heart failure which was accompanied by a sudden fall in sodium excretion and in glomerular filtration rate

It is hoped to elucidate further the mechanism of sodium excretion in renal failure by other experiments to be published later

2   POTASSIUM EXCRETION

When the excretion of potassium is studied by the same methods and in the same type of case the results in general are similar (Fig 3) but since the

percentage of the potassium load excreted is normally much greater than that of the sodium load, and the same kind of relationship exists between the percentage of potassium excreted and the height of the serum creatinine, we find rather unexpectedly that in cases of severe renal failure the percentage of the potassium load excreted is greater than 100, and in occasional cases is in the region of 200 or even 300   This was also noticed by Leaf and Camara (5) using creatinine clearances, and has been confirmed in three of the cases (*see* Table II), in which inulin as well as creatinine clearance was estimated   This can mean one of two things   either potassium is being actively excreted by the tubules, which is the probable explanation, and which, if true, would provide further evidence that the tubule cells have not lost their function in renal failure , alternatively inulin and creatinine are being reabsorbed in these cases and therefore their clearances no longer represent the glomerular filtration rate   There seems to be no good reason for thinking that a large molecule such as inulin should be reabsorbed by the tubule cells in cases of renal failure and it seems more likely, since these cases can remain in equilibrium as far as serum potassium is concerned, for many months, that a mechanism can be brought into play when necessary for the tubular excretion of potassium   A dilated tubule might for reasons already stated have difficulty in reabsorbing some of the solutes passing through it but the same difficulty would not apply to the excretion of substances from the peritubular capillaries provided that the tubule cells were still able to function   The fact that sugar never appears in the urine, even in cases of advanced renal failure, is proof that at least one of the functions of tubular epithelium is preserved

Keith, King and Osterberg (4) have produced a potassium clearance greater than that of inulin by giving large doses of potassium to dehydrated persons, and McCance and Widdowson (6) noted a similar occurrence in a case of renal insufficiency due to alkalosis   The excretion of potassium load in their case was 124% on admission and 18 5% after recovery   The inulin clearance at the same time increased from 9 3 to 55 7 ml per minute

Statistical analysis   The potassium excretion may be considered statistically in the same way as the sodium but the relation to serum creatinine is much closer $\bar{F}$ for the 24 cases of the main series in which K was estimated is 8 6 (standard error 0 190) so that —

Mean % K excretion $= 8\ 6 \times$ (serum creatinine in mg per 100 ml)

This is shown by the heavy line in Fig 3, which also shows the area covered by the standard deviation of $\pm$ 0 93   The 3 cases with heart failure did not have lower potassium excretions than the others, but in the cases with the lowest sodium excretion, potassium estimations were not made   Two of the four cases which had unexpectedly high sodium excretion were found to have a similarly high potassium excretion out of line with the other cases   These have not been included in the analysis

TABLE II

	C/In	C/Cr*	C/Na	C/K	C/P	C/Urea	Serum Na (mEq/l)	Serum K (mEq/l)	Serum Cr (mg/100 ml)	Serum Urea (mg/100 ml)	Serum P (mg/100 ml)	Na % (In)	Na % (Cr)	K % (In)	K % (Cr)	P % (In)	P % (Cr)
JB Period 1	3 00	5 0	0 53	10 5	2 7		134	3 8	10 6		14	13 4	10 6	264	210	68	54
Period 2	2 14	3 0	0 34	6 5	1 8		133	3 8	19 8	705	14	15 8	11 3	304	210	84	60
BW 1	4 15	7 25	0 34	5 35	3 2	2 6	137	4 7	5 5	164	7 8	8 2	4 7	129	75	77	44
2	3 05	0 25	0 3	0 25	3 4	2 5						7 6	4 8	158	100	86	55
3	3 0	4 05	0 26	4 1	3 0	2 3						7 1	5 2	114	83	83	61
FE 1	11 0	13 7	1 0	9 0	6 7	6 2	133	4 0	4 8		7 4	8 5	7 4	83	72	56	49
2	10 6	12 7	0 89	8 4	5 4	5 0	135	4 0	4 8	172	8 0	8 4	7 0	70	66	51	42 5
MR 1	4 3	5 4	0 54	2 0	2 0	3 7	145	5 2	17 2		6 4	12 5	10 0	216	172	47	37
2	4 2	4 0	0 40	2 0	2 0	3 5	145	5 2	20 2	202	6 4	11 5	10 6	203	192	48	44

* Cr signifies Creatinine

A note on Phosphate clearance in Renal Failure

It is interesting to note that in the four severe cases of renal failure investigated by inulin clearance (Table II) the phosphate clearance approached but did not exceed the inulin clearance   The serum inorganic phosphate was very high although the potassium was normal

Homer Smith (9) has shown that in normal dogs the phosphate clearance approaches, but does not exceed, the inulin clearance, at high plasma phosphate levels, and Pitts and Alexander (8) have shown the same phenomenon in dogs comparing phosphate and creatinine clearances

TABLE III

A J   Malignant hypertension

| Date | Urine Vol /24 hrs | Urine Na (m.Eq /l) | Serum | | C /Creat | % Na excreted |
			Na	Creat		
2 4 49	1380	96	139	6 0	20 ml /min	3 3
NaCl 5 gm	given daily until 8 4 49		(Heart failure)			
8 4 49	568	27	154	8 0		
10 4 49	490	20	—	—	4 6	1 4
11 4 49	470	22	146	9 6	3 1	1 6
14 4 49	470	26	—	—	2 8	2 0

We do not propose to decide on the basis of four cases whether tubular excretion of phosphate may occur in man   We can only say that there is no evidence of such a mechanism here despite an extreme plasma phosphate level in one instance and a potassium clearance three times that of inulin The fact that the serum potassium remains normal while the serum phosphate rises, suggests that the latter is handled in a manner more comparable with the handling of creatinine and urea

SUMMARY

In chronic renal failure patients may preserve over long periods a normal plasma level of sodium and potassium in spite of greatly increased plasma levels for urea, creatinine and inorganic phosphate

A study of 26 cases of renal failure has been made by comparing sodium and potassium clearances with creatinine (and in four instances inulin) clearances

This shows a general relationship between the percentage of sodium escaping tubular reabsorption, and the degree of renal failure   Thus plasma sodium is maintained despite a low glomerular filtration rate, by a reduction in tubular reabsorption

The question whether this increased excretion of sodium is due to tubular insufficiency or due to the continued operation of mechanisms for maintaining plasma sodium level is discussed

In the case of potassium the same general relationship holds, but in some instances the potassium clearance greatly exceeds the inulin and creatinine clearances, suggesting that tubular excretion of potassium may take place   This has not been shown in the case of phosphate

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RENAL FUNCTION STUDIES IN ACUTE TUBULAR NECROSIS

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with technical assistance of Miss B EVANS *
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In the course of developing a method of treating uræmia (7) we have investigated over one hundred patients suffering from anuria or extreme oliguria In some of these, the cause was demonstrated to be acute nephritis, malignant hypertension, obstructions to the urinary tract and suppurative conditions of the kidney These do not call for discussion under the above title In a large number of patients, however, other primary conditions were responsible for the renal failure   These included anuria following such causes as ingested poisons, mismatched blood transfusion, abortion, concealed accidental hæmorrhage, "shock," surgical operations, etc It was of interest that all the latter types of anuria presented a uniform pattern of disturbed renal function characterised by evidence of gross tubule disfunction and extreme diminution in renal blood flow The uniformity of functional disturbance was paralleled in the fatal cases by a characteristic pathological picture The term "acute tubular necrosis " is used in this paper to include the whole group This is a descriptive pathological term and avoids the unsatisfactory word nephrosis as was used by Lucké (18) who in 1946 proposed " lower nephron nephrosis " for the condition here being considered Anatomically the characteristic lesion is a necrosis and subsequent regeneration of the renal tubular epithelium The incidents that may lead to this picture include abortion, muscle crushing injuries ("the crush syndrome ") (10), intravascular hæmolysis, various poisons and prolonged renal ischæmia from any cause It is fully appreciated that episodes of tubular disfunction may occur without necrosis of the renal epithelium , such disfunction would not necessarily be accompanied by oliguria In his review, Lucké (18) estimated the mortality of the " lower nephron nephroses " at 90%, although this was in no way related to a failure of the tubule cells to regenerate, but rather to a failure of the patient to survive sufficiently long for renal function to have recovered to some extent

Various hypotheses have been advanced to explain the pathogenesis of different ætiological types of the acute tubular necrosis group Many of these have been based on the pathological findings alone Others involved

speculation without any significant functional data   The present report deals with the pattern of disturbed renal function in acute tubular necrosis and the significance of these findings in relation to the pathogenesis of the condition

Material and methods

Thirty-four patients were investigated and are listed in Table I , certain illustrative cases are described in detail in an appendix (pp 395-404)

TABLE I

Patients investigated

Patient	Age	Sex	Diagnosis
64495	38	F	Anuria following abortion
85878	27	F	,, ,, ,,
86349	27	F	,, ,, ,,
90510	24	F	, ,, ,
92489	20	F	,, ,, ,,
93943	27	F	,, ,, ,,
94135	16	F	,, , ,,
MP	26	F	,, ,, ,
80753	23	F	Anuria following hæmolytic blood transfusion reaction
85006	26	F	,, ,, ,, ,, ,, ,,
85671	34	F	,, ,, ,, ,, , ,,
88570	42	F	,, ,, ,, , ,, ,
89088	32	F	,, ,, ,, ,, ,, ,,
93105	25	M	,, ,, ,, ,, ,, ,
98611	70	M	,, , ,, , ,, ,,
99107	49	F	,, ,, ,, ,, ,, ,,
109762	41	F	,, ,, ,, ,, ,, ,,
BW	66	M	,, ,, ,, ,, , ,,
92068	39	F	Anuria following hæmolytic blood transfusion reaction or severe post hæmorrhagic shock
93123	39	F	,, ,, ,, ,, ,,
87686	52	F	Anuria following post operative shock
106031	59	F	,, ,, ,, ,, ,,
T/QC	33	F	Post hæmorrhagic shock with subsequent anuria
82563	30	F	Anuria following concealed accidental hæmorrhage
MR/183	41	F	,, ,, ,, ,, ,,
T/CM	30	F	,, ,, ,, ,, ,,
93980	41	F	,, ,, ,, ,, ,,
98935	22	F	,, ,, ,, ,, ,
N/T	33	F	, ,, ,, ,, ,,
H/P	31	F	Pregnancy toxæmia, cæsarean operation, operative shock, anuria
89195	36	F	Anuria following mercury poisoning
89964	21	F	,, ,, ,, ,,
76182	54	F	Anuria following ? myanesin poisoning
97194	47	M	Anuria following prolonged vomiting and diarrhœa No response to correction of extra renal factors

It was necessary to obtain data on the renal blood flow, the glomerular filtration rate and tubule function   In the main, standard procedures were used and are listed in the appendix on methods (pp 393-395)   It became clear that in the conditions being investigated, tubule function was defective at certain stages of the illness   This made the ordinary clearance procedures for determining renal blood flow and glomerular filtration invalid   However, it was possible by a more elaborate technique involving the sampling of blood from the renal vein by means of a venous catheter and the simultaneous determination of clearances (31), to obtain satisfactory observations on the renal blood flow (*see* p 394)   When this technique is used, both parenchymal blood flow and blood flowing through other intra-renal channels or shunts is included in the figure obtained
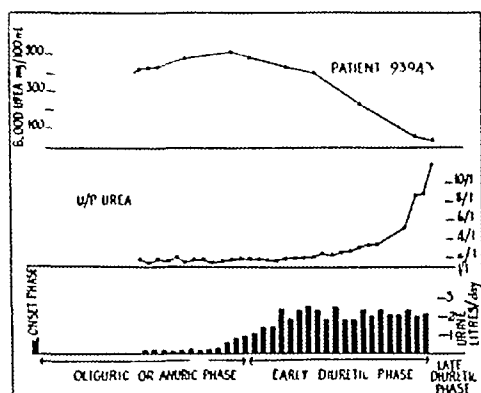

Fig 1   Data on a patient suffering from acute tubular necrosis illustrating the different phases   Low values of U/P urea indicate tubule disfunction (*see* later)   The continuing poor tubular function after the onset of diuresis is shown and is the hallmark of the early diuretic phase

RESULTS

A   *The course of acute tubular necrosis*

An essentially similar pattern of disturbed renal function was found in all cases, despite varying primary causes for the anuria or extreme oliguria   The causal states naturally coloured the clinical picture, but, despite this, all cases ran a similar course   Other workers have clearly appreciated the fact that the course of the disease can be divided into phases that have differing clinical features and patterns of renal disfunction (21)

After an *onset phase* such as a period of severe and prolonged " shock " or a period during which a toxin such as mercury is acting, there follows a *phase of anuria or severe oliguria* (here defined as less than 300 ml urine per day) which lasts from a day to as long as three weeks   In patients who recover, the anuric or oliguric phase is followed by a gradual, or, less commonly, a sudden increase in daily urine volume   When the daily urine volume exceeds 1 litre, the patient is said to be in the *early diuretic phase*

During the early days after the re-establishment of urine flow, tubule function is virtually absent but recovers after a further period   When evidence of returning tubule function is obtained, the patient is considered to have passed into the *late diuretic phase*   Fig 1 illustrates the various phases in a patient suffering from acute tubular necrosis following self-induced abortion

The data presented deal with patients suffering from acute tubular necrosis of all types investigated   In the appendix (pp 395-404) examples of acute tubular necrosis of varied ætiology are presented in detail

B   *Tubule function*

Tubule function has been studied in four ways —

1   *The ability of the tubules to concentrate waste products* is shown by the capacity of the kidney to raise the concentration of urea or creatinine to levels greater than in the blood   This is expressed as the ratio —

$$\frac{\text{Urine concentration of urea or creatinine}}{\text{Plasma concentration of urea or creatinine}} = \text{U/P urea or creatinine}$$
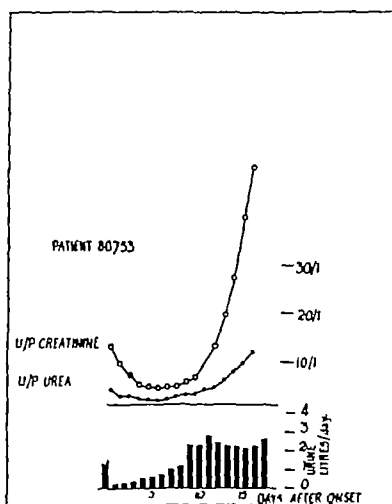

Fig 2   The ability of the kidney to concentrate urea and creatinine in the urine in acute tubular necrosis as illustrated by the findings in patient 80753
    The low figures for the ratios urine/plasma urea and creatinine indicate poor tubule function   The delay in the return of tubule function after the onset of diuresis is typical

In health, these ratios may exceed 100/1 especially when the urine flow is low   With severe depression of tubule function, ratios of 1/1 will be approached whatever the rate of urine flow   Fig 2 shows the behaviour of these ratios in an illustrative case

It will be seen that the U/P ratios for urea and creatinine approach 1/1 indicating very severe tubular damage   Furthermore, tubule function recovers some time after the onset of a diuresis   This continuation of the

defective tubule function after the onset of diuresis characterises the early diuretic phase   Table II shows the U/P ratios for urea and creatinine in other patients

<div align="center">

TABLE II

*The ratios of urine/plasma concentrations of urea and creatinine
in patients suffering from acute tubular necrosis*

</div>

Patient	Mean ratios in oliguric phase		Ratios on day of 1 litre diuresis	
	Urea	Creatinine	Urea	Creatinine
80753	1 8	7 9	1 8	4 3
90510	1 5	3 9	1 4	4 8
92008	1 5	1 4	1 4	0 9
93943	1 6	3 1	1 8	3 4
94135	1 7	2 1	2 3	2 3
80964	—	—	0 9	—
80195	—	—	1 6	—
106031	2 0	9 6	3 3	—
98011	2 9	—	—	—
86349	1 2	—	1 8	—
91794	3 1	—	1 8	—
89088	0 8	—	—	—
99107	1 7	—	1 7	—
85071	—	—	2 4	—
T QC	2·0	—	1 9	—
MR184	3 2	—	—	—
109762	1 2	—	1 3	—
N/T	2 2	—	1 5	—
Mean	1 93	4 66	1 78	3 14

These low U/P ratios indicate that severe tubular disfunction occurs regularly in this condition

2   *The ability of the tubules to conserve ions*   When *low* plasma levels of sodium, chloride or potassium are present, normal kidneys meet the body's requirements for these ions by actively re-absorbing them from the glomerular filtrate so as to produce high plasma/urine (P/U) ratios for these substances   Indeed a normal kidney can elaborate urine so low in chlorides (high P/U ratio) as to contain less than many samples of tap water   In certain of our patients, the plasma levels of sodium and chloride were well below the normal levels, yet, in all these cases, the urine contained considerable quantities of sodium and chloride   This is a further indication of gross tubular disfunction   Fig 3 shows the findings in a typical case

Considerable tubule disfunction thus demonstrated, persists for an appreciable time after the onset of diuresis   It will be understood that if there is sufficient daily intake of these ions to meet the body's requirements or if the plasma level of the ions is normal, these ratios cannot be used to demonstrate tubule disfunction   The fall in P/U ratio for chloride on day 28 in the figure does not represent a recurrence of tubule disfunction but

merely indicates that the body requirement for chloride has been met
Table III shows the P/U ratios for sodium and chloride in a number of
patients in whom the conditions permitted the demonstration of tubule
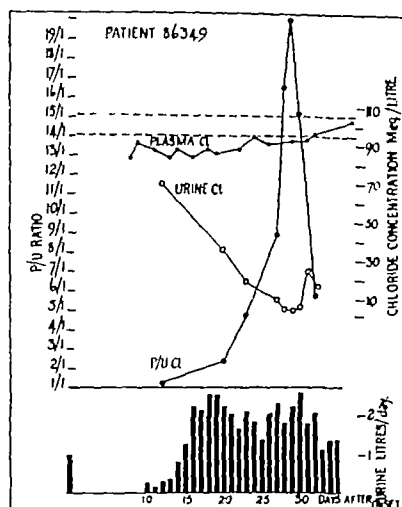disfunction

Fig 3   The ability of the kidney to conserve chloride ion under conditions where this con
servation should normally occur in a patient with acute tubular necrosis following mercury
poisoning
        For interpretation see text

TABLE III

*The ratios of plasma/urine concentrations of sodium and chloride
in patients suffering from acute tubular necrosis*

Patient	Mean ratios in oliguric phase		Ratios on day of 1 litre diuresis	
	Sodium	Chloride	Sodium	Chloride
80753	3 0	3 3	3 4	3 8
90510	2 2	2 0	2 4	2 1
92068	2 6	2 3	2 3	2 4
93943	1 8	1 9	1 5	1 2
94135	1 7	1 8	2 2	2 8
89064	—	—	1 6	1 3
89195	—	—	1 0	1 4
98611	1 6	1 8	—	—
86349	—	1 2	—	—
91794	—	—	—	2 0
89088	—	1 3	—	—
99107	—	—	—	1 5
85671	—	—	—	1 3
T QC	—	1 2	—	3 5
109762	1 4	1 4	1 5	1 4
N/T	—	—	—	1 3
Mean	2 05	1 82	1 95	2 0

Distuibance of tubular regulation of ion concentration can be shown in another way In the figure on page 399 the sodium and chloride intake and output in acute tubular necrosis are plotted During the early diuretic phase, the patient was in conspicuous negative sodium and chloride balance despite abnormally low blood levels of these ions

Similarly, the tubular reabsorptive capacity for potassium under conditions of potassium lack was shown to be defective in three patients in whom the point could be examined The figure on page 400 shows data on one of these In another patient, potassium loss was sufficiently severe to cause a flaccid paralysis which responded to potassium administration

3   *The extraction of para-amino-hippurate from the blood by the kidney*
At low levels of para-amino-hippurate in the arterial blood, the kidneys normally remove between 85 and 100% from the blood flowing through them , that is, the extraction of para-amino-hippurate (E_{PAH}) is 85–100% E_{PAH} was estimated in several patients at various stages of the illness In each case the arterial level of PAH was below 3 mg per 100 ml   As PAH extraction is a tubular function, low figures indicate tubule disfunction , renal vascular shunts might similarly lower E_{PAH} but this factor is excluded on other grounds (*see* p 394)   Fig 4 shows the results of estimations of E_{PAH} in 7 patients
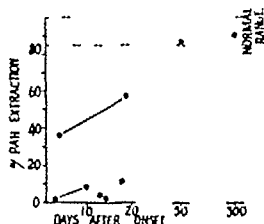

Fig 4   The percentage of renal extraction of para amino hippurate in patients suffering from acute tubular necrosis
The low values for E_{PAH} indicate tubular disfunction   The extraction improves with time after the onset of the condition

4   *Tubular reabsorption of glucose*   The amount of glucose reabsorbed can be estimated and in normal women is 303 $\pm$ 55 3 mg per minute (13) This is known as Tm_G and low values indicate a diminution in the functional capacity of the proximal convoluted tubule   In two patients we determined Tm_G and the following results were obtained —

In patient 82563 on the 19th day after onset, the Tm_G was 107 mg per minute when the simultaneous glomerular filtration rate was 45 ml per minute and para amino-hippurate clearance was 224 ml per minute

In patient MR 181 Tm_G was 213 mg per minute when the simultaneous glomerular filtration rate was 74 ml per minute and the para-amino-hippurate clearance was 266 ml per minute

In both patients the Tm_G was low   In several patients in whom the blood sugar was not artificially raised, we attempted to demonstrate glycosuria but were able to prove its presence in one case only

The duration of tubule disfunction

The various tubule functions return at slightly variable intervals after the onset of diuresis   The special risks resulting from tubular disfunction in the early diuretic phase become negligible when tubule function has recovered to the point at which the following ratios are found —

U/P urea   10/1
U/P creatinine   20/1   -
P/U sodium and chloride   5/1

The clinical duration of the early diuretic phase may be estimated by taking the mean period for recovery of these functions   Table IV shows the duration of the oliguric and early diuretic phases in patients with acute tubular necrosis

TABLE IV

The duration of the oliguric or anuric and early diuretic phases
in patients suffering from acute tubular necrosis

Patient	Duration of oliguric or anuric phase in days	Duration of early diuretic phase in days
86349	14	16
90510	17	17
93943	23	16
94135	14	9
80753	6	8
92068	11	9
106031	7	5
T QC	12	10
T	7	15
89195	11	12
80964	12	11
109762	9	10
N/T	12	18
Mean	11 8	12 0

This shows that the duration of the early diuretic phase is approximately the same as the anuric or oliguric phase that precedes it   From the point of view of treatment, the special dangers of the early diuretic phase are uncontrolled mineral and water loss   Careful control of mineral and water intake is therefore necessary after the onset of diuresis for a period as long as the preceding oliguric phase

C   *Renal plasma flow*

When the tubule function was defective as shown by the tests indicated above, the renal plasma flows were estimated by the procedure outlined on p 394, which involved the estimation, by means of renal vein catheterisation, of the extraction of various substances and their simultaneous clearances   Where tubule function was normal, as occurred later in the course of the illness, the ordinary clearance procedure (p 394) was used   That this latter method was valid may be assumed from the fact that the extraction of para-amino-hippurate rises progressively from the onset

(p 385) Table V shows the renal plasma flow in a patient estimated by the clearances and extraction ratios of three substances observed simultaneously

TABLE V

Renal blood flow on the 10th day after onset of acute tubular necrosis calculated from renal clearances and extractions of three substances

Substance	Patient 93943				
	Renal extraction %	Plasma clearances ml per min			Renal plasma flow ml per min. Mean
		Period 1	Period 2	Mean	
Para amino hippurate	2 11	0 27	0 47	0 37	17 5
Creatinine	1 38	1 38	1 74	1 56	34 1
Urea	0 96*	0 22*	0 25*	0 24*	18 8†
Average					
Corrected to 1 73 sq metres surface area					23 5 ± 9 8
Whole renal blood flow (packed cell volume 23%)					27 5
					35 7

* Calculated from whole blood not plasma
† Calculated from whole blood clearance allowing for packed cell volume 23%

The figures show that the renal blood flow was extremely low and the estimates obtained from the three substances show fair agreement considering the difficulties involved with the low extractions

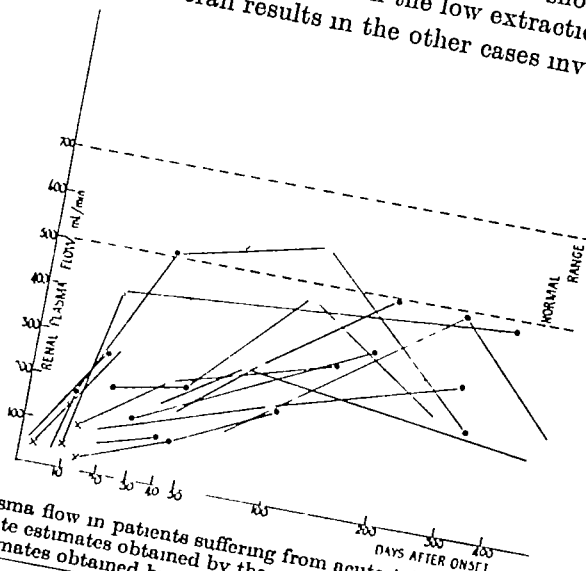Fig 5 shows the overall results in the other cases investigated

Fig 5 The renal plasma flow in patients suffering from acute tubular necrosis
Crosses indicate estimates obtained by the method involving renal vein catheterisation *
dots indicate estimates obtained by the standard method

* These results have been calculated from the formula — $\dfrac{UV}{A-R}$

Where the extraction ratio is very low, the formula — $\dfrac{UV}{A-R} - \dfrac{VR}{A-R}$ should be used as was pointed out in a footnote by Wolf (32) Using this formula in these cases would further reduce the plasma flow by at most 30%

D   *Renal oxygen consumption*

In a number of cases the renal blood flow was estimated by the method involving renal vein catheterisation (p 394)   The oxygen content of arterial and renal venous blood was estimated at the same time and from the data, the oxygen consumption of the kidney was calculated   Table VI shows the findings

TABLE VI

Oxygen unsaturation of arterial and renal venous blood and renal oxygen consumption in patients suffering from acute tubular necrosis

Patient	Day of illness	A Arterial blood ml per l	RV Renal vein blood ml per l	A–RV	Oxygen consumption ml per min
89964	29	0 3	25 4	16 1	2 2+
93943	14	9 0	43 0	34	1 21
93068	13	4 0	19 0	15	2 03
80753	3	13 0	47 0	34	2 13
	10	9 0	43 3	34 3	6 21
98935	3	9 5	35 0	25 5	5 6
Normal*				10 8 (6–16)	10 3 (6–14 2)

* Quoted from Bradley and Halperin (4)
   Five normal subjects we have investigated gave figures within the same range

The figures for oxygen consumption per minute are only approximate because of the inaccuracy of the renal blood flow determinations at these low levels   However, the high renal arterio-venous oxygen differences are of importance (*see* discussion)

E   *Glomerular filtration rate*

Where tubular disfunction exists it is impossible to guarantee that thiosulphate or inulin clearances necessarily represent the true glomerular filtration rate in that these substances may be passively reabsorbed through non-functioning tubules   However, when tubular functional recovery can be demonstrated, it is probable that these clearances become correct indices of glomerular filtration   Fig 6 shows the estimates of glomerular filtration obtained

It will be seen that the thiosulphate clearances are extremely low in the early stages and that they increase in parallel with the improvement already shown in renal plasma flow (p 387)   One case showed a significant fall in the glomerular filtration rate in the late follow up period

Endogenous creatinine clearances estimated from 24 hour urine collections are shown in Fig 7

The results roughly parallel the findings from the thiosulphate clearances

Even if the thiosulphate and creatinine clearances are not valid indices of glomerular filtration, this function must be very grossly diminished because of the very low rates of renal blood flow
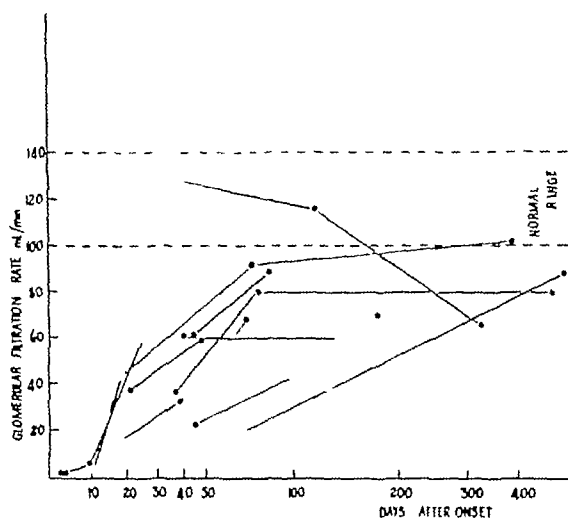

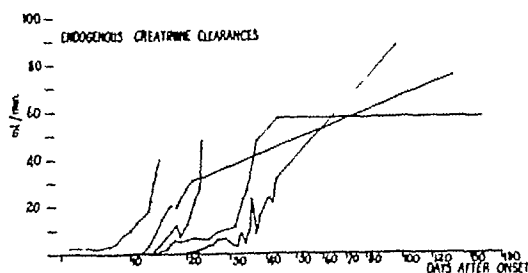Fig 6   Thiosulphate clearance data on patients suffering from acute tubular necrosis

Fig 7   Endogenous creatinine clearance data on patients suffering from acute tubular necrosis

F   *Miscellaneous observations*

In several patients, cardiac output determinations were made and found to be within normal limits

In patients 82563, 86349, 90510, 91794, 93105, 72969, 72174, splanchnic block or spinal anæsthesia failed to alter the course of the disease

INTERPRETATION OF FINDINGS

The observations made cover the period from the oliguric or anuric phase until just over a year from onset   Certain conclusions regarding function during this time can be drawn from the data presented   No observations could be made in any of our cases during the " onset phase "

Tubule function diminishes rapidly during the first few days (approx 5 days in a mild case and more rapidly in a severe case) of the illness, becoming virtually absent and then gradually improving during the following weeks A point of great importance in therapy is the fact that the return of tubule function is delayed for days or weeks after the onset of diuresis As a result of this, *the early diuretic phase is characterised by an outpouring of essential electrolytes which if not balanced by an adequate dietary intake, may cause the death of the patient* Our demonstration of the disturbed tubular mechanisms is supported by the work of Burnett *et al* (8) on the specific gravity of the urine in this condition and the dangers of the early diuretic phase have been previously recognised (9, 28)

The affection of the tubule seems to be general as we have been unable to demonstrate any tubule mechanism which has escaped The absence of glycosuria in most patients does raise the possibility that the proximal convoluted tubule is relatively spared but other explanations of this phenomenon are more probable The point is to be further investigated

Renal blood flow The observations of the renal blood flow made by the Fick procedure involving renal vein catheterisation are of particular importance Determinations made by this method include in the estimate of blood flow, any blood passing through an intra-renal shunt It can be confidently stated, therefore, that in the oliguric phase, the renal blood flow through parenchyma, including any possible shunt, is very grossly reduced to levels of 10% of normal or below This finding is very strong evidence against any significant shunt being in operation at the time Experimentally induced shunts in animals are associated with rates of renal blood flow of over 50% of normal (1, 12) The high renal arterio-venous oxygen difference in our cases provides further evidence against participation of a shunt of any magnitude Trueta *et al* report that in experimentally induced shunts in animals, the renal vein contains " bright red blood " (29)

There is, therefore, a real and gross reduction of the renal blood flow in the oliguric phase and the flow tends to rise steadily thereafter About 12 to 20 weeks after onset it is slightly below normal or occasionally normal In some cases there is a subsequent fall about a year later, a point which is to be further investigated

Glomerular filtration is reduced in parallel with the reduction in renal blood flow but no certain conclusions can be drawn on the filtration fraction

Sirota (27) using similar techniques to our own has made observations of the renal blood flow and renal oxygen consumption in patients with carbon tetrachloride poisoning and his findings agree with ours No other observations on the renal blood flow in the oliguric or early diuretic phases of the illness appear to have been made previously, but the pattern of returning blood flow in the later phases has been reported (19, 2), with results similar to our own.

Function in the onset phase Although we were unable to make observations during the onset phase in our patients, the probable type of disturbed function at that time can be inferred from the following —

There is ample evidence to show that traumatic, post-hæmorrhagic and other varieties of " shock " in animals and man are accompanied by a gross diminution in renal blood flow (30, 17) " Shock " is a frequent accompaniment of the onset phase of certain types of acute tubular necrosis, e g , crush syndrome, anuria following concealed accidental hæmorrhage and the " shock kidney " of van Slyke (30) Therefore, a primary renal ischæmia must exist in many cases, which, if the general circulation is improved by appropriate measures, is reversible (30) However, when this primary ischæmia has merged into the secondary ischæmia of the oliguric phase, it becomes refractory to all such therapy
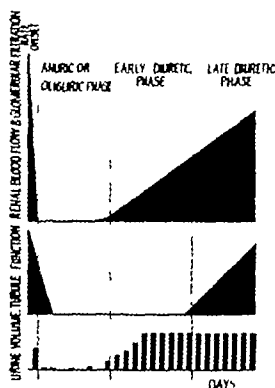

Fig 8   The overall pattern of disturbed renal function in acute tubular necrosis
        Return of tubule function is delayed for an appreciable time after the return of glomerular filtration and urine flow

Certain types of acute tubular necrosis are probably not associated with a primary ischæmic phase   It has been found in experimentally induced pigment " nephrosis " in animals that the renal blood flow was normal immediately following the injection of hæmoglobin but it fell off gradually during the next three hours (14)   In the mildest form of mercurial tubular disfunction following the injection of a therapeutic dose of an organic mercurial, there is no significant alteration in the renal blood flow or glomerular filtration rate (6)   Large doses of mercury, however, lead to the full picture with superadded secondary ischæmia (*see* patients 89195, 89964)

The probable overall pattern of disturbed renal function in acute tubular necrosis is shown in Fig 8

Discussion

The uniform functional disturbance is paralleled by a characteristic histological appearance details of which will be published by Professor Dible   This suggests that acute tubular necrosis is a reaction pattern to a wide variety of acute renal insults   A full discussion of this concept would involve consideration of an extensive literature and will be reserved for a future paper   We would suggest, however, that there are two main types of acute tubular necrosis, one resulting from poisoning of the kidney cells and the other from very severe renal ischæmia   In either case, once the condition is established, recovery is possible only by a regeneration of damaged tubule cells   Diuresis almost always should occur if the patient survives the period of oliguria   In one of our patients urine flow recommenced as late as the 23rd day   Treatment should, therefore, be directed towards keeping the patient alive for this period at least

From a consideration of our findings, certain conclusions can be drawn regarding possible therapeutic procedures —

1   Any general circulatory disturbance which leads to renal ischæmia should be corrected as rapidly as possible

2   As glomerular filtration is virtually absent in the oliguric or anuric phase, water, minerals and other substances cannot be excreted in the urine and will accumulate if fed in excess of the amounts that can be excreted or lost by other routes   For the same reason, diuretics which act either osmotically or by diminishing tubular water reabsorption cannot be effective at this time and indeed will be harmful

3   Once anuria or oliguria is established, splanchnic block or spinal anæsthesia will not alter the course of the disease

4   In the early diuretic phase, the urine has the composition of a very slightly modified glomerular filtrate and minerals are lost in the urine regardless of body needs   Therefore, during this phase, it is vital to ensure an intake of various ions which will balance the urinary loss   The concentration of sodium and chloride in urine at this time is approximately half that in the blood (mean P/U ratio 2/1 *see* p 384) and adjustment of the dietary salt intake on the basis of this figure will maintain the patient in approximately normal salt balance   No simple rule can be derived from the data to calculate the potassium needs of the patient in the early diuretic phase, but in practice we have found that patients fed on a diet largely composed of fruit from the beginning of this phase seldom develop clinical evidence of disturbed potassium metabolism

5   The early diuretic phase lasts on the average as long as the oliguric or anuric phase which precedes it   During this whole period, accurate control of water and mineral intake will be necessary

SUMMARY

Thirty-three patients suffering from anuria or severe oliguria following the ingestion of poisons, intra-vascular hæmolysis, shock, abortion, concealed accidental hæmorrhage and prolonged and severe " extra-renal " uræmia have been investigated   Whatever the primary cause, they ran a similar clinical course and showed the same disturbances of renal function   The course can be divided into four phases

1	Onset	2	Anuric or oliguric
3	Early diuretic	4	Late diuretic

No opportunity arose to study the onset phase in these patients but in the other phases, tubule function, renal blood flow, renal oxygen consumption and glomerular filtration were studied

Tubule damage was shown in the second and third phases by inability of the kidney (1) to concentrate urea and creatinine, (2) to conserve sodium, chloride and potassium, (3) to extract para-amino-hippurate from the blood and (4) to reabsorb glucose at a normal rate

Renal blood flow, measured by para-amino-hippurate clearance, checked where necessary by renal vein catheterisation, was grossly reduced during the oliguric phase and steadily increased from then on, until it reached nearly normal levels in a period varying from 3 to 9 months

Glomerular filtration, as measured by thiosulphate clearance showed reductions of the same order as the renal blood flow

The renal arterio-venous oxygen differences were increased when the renal blood flow was low   This and other findings excluded the presence of an intra-renal shunt of any magnitude during the phases investigated

The term " acute tubular necrosis " is used to include all the conditions studied and it is regarded as a reaction pattern of the kidney to a variety of acute renal insults   Two main types, toxic and ischæmic, are distinguished

The significance of the functional findings in relation to treatment are discussed

APPENDIX 1

Methods

1   *Urine collections* are made over 24 hour periods from 10 a m to 10 a m and recorded for the day on which collection begins   Clean Winchester bottles containing about 5 ml of toluene are placed beneath the bed by the technician and all urine passed is immediately added to the collection   When urine is inadvertently passed with fæces it is filtered off and added to the collection   The full bottle is collected by the technician and its contents measured   No measurements are made by the nursing staff   This technique was suggested to us by Prof Borst (3)

2   *Blood samples* are drawn in dry sterilised syringes and heparinised   An aliquot is retained for determination of the packed cell volume and the remainder centrifuged immediately The plasma is separated immediately and used for all biochemical determinations

3   *Renal clearance techniques*   Samples of venous blood and urine (by urethral catheter) are obtained   The following injections are then given —

   (i) 10 gms Anhydrous sodium thiosulphate, dissolved in 20 ml pyrogen free distilled water and sterilised by boiling, injected intravenously, slowly or 50 ml 10% solution of pyrogen free inulin (Kerfoots) injected subcutaneously into the axilla

   (ii) 1 gm Para amino hippuric acid (PAH) as sodium salt dissolved in 5 ml pyrogen free water and sterilised by Seitz filtration or boiling injected subcutaneously with 1–2 ml " Stovaine " or " Percaine " (not " Novocaine ' or ' Procaine ")

Twenty to thirty minutes is allowed to pass for equilibrium to be established between plasma and extra-cellular fluid (complete equilibrium cannot occur but variation greater than 10% in venous blood level of PAH or inulin seldom occurs over a period from 20 mins to 1 hour after the injection, especially where renal function is poor)   The bladder is then drained of urine, 20 to 50 ml of sterile, distilled water and up to 200 ml of air are injected into the bladder and then expressed by supra pubic pressure   This procedure is repeated once   Immediately thereafter, urine collection is begun and continued until at least 50 ml of urine are obtained The bladder is then emptied in the same way and the washings added to the urine collection The collection period is timed to the nearest half minute, the urine measured and an aliquot is retained for analysis   A second collection of urine is made over a further consecutive period Blood samples are obtained after each bladder emptying and estimated for thiosulphate, PAH, urea and creatinine, along with the urine from the two collection periods   By intrapolation, blood levels of these substances are estimated for the " corrected midpoint " of each collection period   This correction is made to allow for the time taken for urine to traverse the dead space of the ureters and renal pelves and is calculated as follows   When the urine flow is 10 ml per minute, one minute is subtracted from the true midpoint   When the urine flow is 5 ml per minute, two minutes are subtracted from the true midpoint, etc

Clearances are calculated from the formula UV/P where U is urine concentration in mg per 100 ml , P is plasma concentration in mg per 100 ml and V is urine volume per minute

(*Note* —Thiosulphate, PAH and endogenous creatinine clearances are expressed as clearances of plasma and urea clearance as of whole blood)

The patient is kept in bed throughout the study period and is only allowed water to drink for one hour before

   (a) Para amino hippurate estimations and interpretations of clearances as measuring effective renal plasma flow (13)

   (b) Thiosulphate estimations and interpretation of thiosulphate clearance as measuring glomerular filtration rate (22)

        Modification —Urine is diluted suitably so as to have approximately the same concentration of thiosulphate as the protein free plasma filtrate and estimated in the same way as the filtrate

   (c) Urea estimations for clearances—urease—Nessler method (16)

   (d) Creatinine estimations (23)

        Modifications —Urine is not corrected to specific gravity of 1 01 before making 1/10 to 1/50 dilutions and the procedure is adapted for use of 2 ml plasma samples

        Interpretation of endogenous creatinine clearance as an approximate test of glomerular filtration rate (5)

The coefficient of variation for duplicate or replicate clearances using this technique in 100 consecutive patients in this clinic was —

15 4% for para amino hippurate
23% for thiosulphate

4   *Renal blood flow determinations in the presence of tubular disease*   Where tubular function is deficient, the above technique is inapplicable because it depends on the assumption that the blood flowing through the kidney is almost completely cleared of para amino hippurate However, if the extraction ratio for any substance—

$$E = \frac{\text{Arterial blood concentration} - \text{renal venous blood concentration}}{\text{Arterial blood concentration}} \times 100$$

is known as well as its clearance, the true renal blood flow can be calculated (31)   Therefore, the clearance and extraction ratio of one or more of the following four substances was calculated simultaneously—PAH, thiosulphate, creatinine and urea   In the case of the exogenous substances PAH and thiosulphate, the dose of the substance was given not less than 60 mins before the start of the clearance period   This was done because, at the time the clearances were estimated, the urine flow was extremely low and it was necessary to allow a very long period for the urine to traverse the dead space of the renal pelves and ureters before commencing timed collections   The midpoint of each clearance was calculated as before

During the clearance period samples of arterial blood were obtained from the brachial or femoral artery and simultaneous samples of renal vein blood were collected by the technique of renal vein catheterisation (31) Replicate samples were taken, moving the position of the catheter slightly in the renal vein and checking the position of the fluoroscopic screen Due care was taken to discard between 5 and 10 ml of the first blood withdrawn through the catheter before collecting samples for analysis so as to avoid dilution effects due to the dead space of the catheter As a further check on this the hæmatocrit was determined on all samples

It will be appreciated that where this technique is used to determine renal blood flow, both parenchymal blood flow and blood flowing through other intra renal channels or shunts will be included

5 *Determination of oxygen unsaturation of blood* Blood samples are collected anærobically in oiled syringes and analysed within a two hour period The analyses are made on the Haldane blood gas apparatus (24)

6 *Other biochemical determinations*

(a) Chloride in plasma (26) Coefficient of variation in this clinic 2 21%

(b) Chloride in urine (16) Coefficient of variation in this clinic 4 2%

(c) Urea in urine (16) Coefficient of variation in this clinic 4 8%

(d) Magnesium Colorimetric method of Briggs (24)

(e) Ammonia + amino acid nitrogen in urine — formol titration (15)

(f) Total plasma proteins (25)

All other biochemical estimations quoted have been carried out in the routine manner in the Biochemistry Department by standard methods (16) and we are indebted to Prof Plimmer and his staff for the results Sodium and potassium estimations were carried out by the method of flame photometry (11)

7 *Balance studies* Preparation weighing and calculation of oral diets according to standard tables of reference (20) have been carried out with the help of the dietetic staff Synthetic diets have been prepared with the help of the dispensary staff and calculated by ourselves

APPENDIX 2

Illustrative cases

Patient 89904 aged 21 years, single woman a private in the Auxiliary Territorial Services Her medical category was A 1 and she had no relevant previous history

On 5th August, 1948, ' in a fit of depression ' she took an unknown quantity of mercuric oxide Within a few minutes she felt faint and vomited and later complained of abdominal colic and diarrhœa Despite emergency treatment including gastric lavage but not administration of British Anti Lewisite, she became anuric and was transferred to Hammersmith Hospital on the 11th August.

On admission she was a well nourished woman of medium build She had a slight fever, was drowsy and unco-operative There was no evidence of over or underhydration There was a severe stomatitis Chvostek s sign was easily elicited and the plantar responses were extensor but there were no other abnormal signs in the nervous system The laboratory findings are shown in the accompanying figures, 9 and 10

High calorie protein and mineral free feeding was commenced immediately i e, on the 7th day of the illness Further progress can be followed on the charts Diuresis commenced on the 12th day On the 15th day she became confused and developed urinary incontinence Her blood urea which had climbed steeply before the high calory feeding climbed more slowly, reaching its height on the 14th day and then fell The serum potassium fell slowly at first and then more steeply after the onset of diuresis until by the 20th day it was grossly subnormal This fall was accompanied by an increasing weakness and with this she developed tremor, ataxia and dysarthria On the 24th day, after administration of 1 gm of potassium chloride intravenously there was an immediate but slight improvement in muscular power and further increase in power was shown by serial determinations of grip strength with a dynamometer following the oral administration of a further 5 gms of potassium chloride The tremor ataxia and dysarthria became more apparent and were very gross Gradual improvement occurred over the next few weeks

The drowsiness and lack of co-operation persisted until the 27th day and on this day urinary continence returned and her mental state improved

Hypertension was evident from the date of admission No previous recordings of the blood pressure could be found in her army records but it seems improbable that she had hypertension preceding this illness The hypertension was sustained from the 39th day until

the last observation on the 83rd day   The degree and persistence of the hypertension was exceptional and none of our other patients with acute tubular necrosis showed more than transient rises in blood pressure and even that was not the rule   The other case of mercury tubular necrosis showed neither the central nervous disturbances nor the blood pressure rise
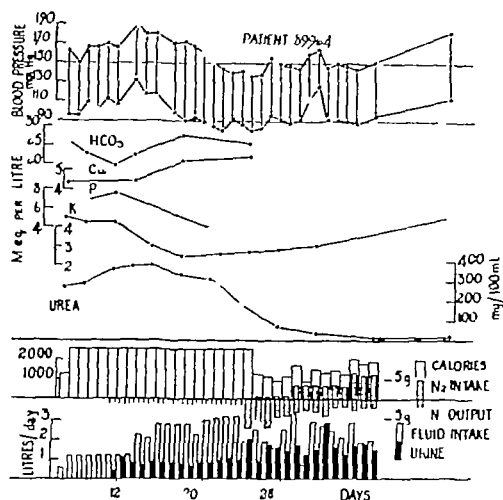

Fig 9

Throughout the course of the illness the urine showed a persistent faint proteinuria and microscopically, small numbers of red and nucleated cells and granular casts were found

A mild pyrexia persisted throughout the first two months of her illness   It could not be accounted for on the basis of either urinary or other infection.   Diarrhoea and stomatitis which only persisted to the 27th day, could not explain it

The poor tubule function which persisted until well after the onset of diuresis is shown by the low ratios for U/P urea and P/U chloride and sodium and can be inferred also from the falling blood levels of sodium, chloride and potassium
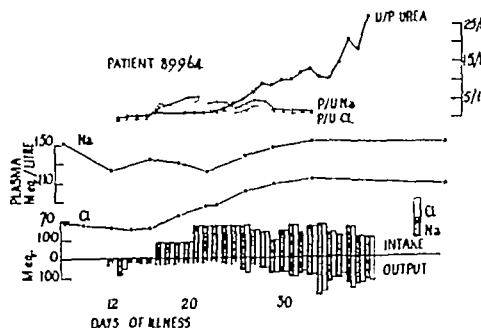
The clearance data show the disturbances in renal dynamics

Fig 10

Conclusion   Acute toxic tubular necrosis of unusual severity due to the ingestion of a mercuric salt   The delayed onset of tremor, ataxia and dysarthria is of interest and was of the type ordinarily seen in chronic poisoning   The time of onset coincided with the maximum disturbance of ion balance

Patient 82563, a married woman of 30 years, was admitted with anuria following a concealed accidental hæmorrhage

In 1945 she had a miscarriage at three and a half months In March, 1946, when 30 weeks pregnant she developed " toxæmia with albuminuria œdema and a blood pressure of 210/130 mm.Hg The pregnancy was terminated In January 1947, she was found to have a blood pressure of 130/85 mm.Hg , blood urea 31 mg per 100 ml and urine urea concentration 3 gms per 100 ml and was advised that she could become pregnant again

She menstruated in the first week of May 1947 and on 11th Nov when she was 30 weeks pregnant her blood pressure was found to be 160/105 blood urea 13 mg per 100 ml and urine urea concentration 3 4 g per 100 ml with protein concentration 20 mg per 100 ml She was admitted to hospital where the blood pressure fell but rose again a week later and heavy albumin uria and œdema developed Hypertension of the order of 170/110 mm.Hg , albuminuria and œdema persisted until 21st Dec when she had a sudden onset of severe abdominal pain and vomiting The uterus was enlarged and tender and the fœtal heart sounds were absent Within twelve hours there was spontaneous labour and normal delivery of a stillborn fœtus The placenta was infarcted and there was a retroplacental clot No urine was passed from the onset of the pain and no urine was obtained on catheterisation A splanchnic block was performed 18 hours after the onset of the pain with a fall in blood pressure from 130/100 to 54/36 mm.Hg but the blood pressure rose again in a few hours

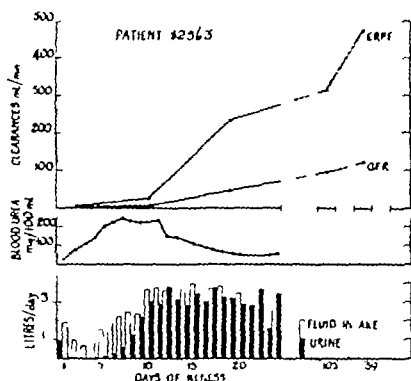
Fig 11

On 23rd Dec she was transferred to Hammersmith Hospital as the anuria persisted On examination she was a well-covered healthy looking, intelligent woman There was no œdema, increased venous pressure or other signs of water overload The blood pressure was 144/90 mm.Hg There was some bilateral renal angle tenderness and the uterus was firm, at the level of the umbilicus and not tender

She was treated with a high calorie non protein diet and fluid intake was carefully controlled The subsequent course can be seen from the chart Fig 11 and renal function data can be seen in the table The blood pressure remained at 140/90 throughout her stay in hospital and was at this level when seen as an outpatient 396 days after the onset of the anuria

Conclusion A case of concealed accidental hæmorrhage complicated by anuria of five days duration and with a course typical of acute tubular necrosis Bilateral cortical necrosis occurs most commonly as a complication of concealed accidental hæmorrhage and in this case the differential diagnosis was impossible except by the subsequent course

Patient 90510 a 24 year old married woman attempted to terminate a third month pregnancy with synthetic œstrogens and having failed, on 28th Aug , 1948, administered to herself an intra uterine douche of soapy water This procedure was followed by faintness and lower abdominal colic which caused her admission to another hospital There she was given two pints of plasma, coramine, penicillin and abundant fluid to drink but she vomited copiously On the next day she was delivered of an immature fœtus and as she had been anuric from the

Renal function data on patient 82563

Days after onset	Para amino hippurate clearance ml per mn	Extraction PAH %	Extraction creatinine %	Renal plasma flow (corrected) ml per mn	Thiosulphate clearance ml per mn	Tma	ERPF/Tma	GFR/Tma
2	0	—	—	—	0	—	—	—
4	—	35 7	6 7	—	—	—	—	—
10	24	—	—	—	3 4	—	—	—
10	224	58 1	22 7	393	45 0	107	2 03	0 42
105	312	—	—	—	95 0	—	—	—
392	475	—	—	—	122 0	—	—	—

time of the douche she was transferred to a second hospital where penicillin and intra venous
fluid therapy were continued. On the day of transfer to the second hospital she was noted to
be jaundiced. High spinal anaesthetic to the level of the 4th thoracic segment was induced on
the 30th Aug, and this was followed by a fall in blood pressure to 80/30 mm Hg. Lower
abdominal cramps, vomiting and anuria persisted and she was transferred to Hammersmith
Hospital on 31st Aug. She had no relevant previous history. There were no previous mis
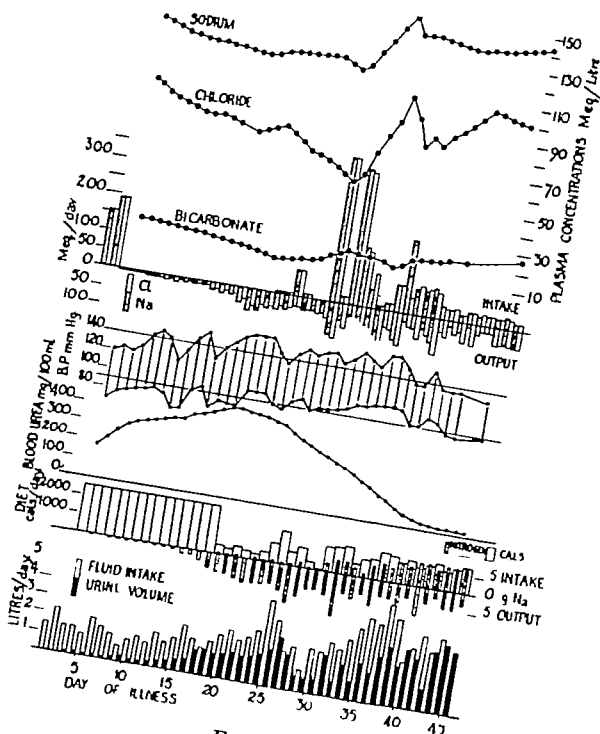carriages and she had two healthy children aged 9 and 23 months respectively

Fig 12

On examination on admission she was alert, anxious, of medium build and normal complexion
The pulse rate was 94 per minute, temperature 98°F, and respiration normal in type and 18
per minute. Eyeball tension was normal, tongue clean and moist. skin of normal turgor and
there was no oedema. The jugular venous pressure was below the sternal angle and the blood
pressure was 120/68 mm.Hg. There were no abnormal findings in the cardio vascular, respiratory
or central nervous systems. The electrocardiograph was normal. She had no renal angle
tenderness but there was diffuse abdominal tenderness rendering palpation difficult. Vaginal
examination showed that the cervix was dilated to one finger's breadth and contained conception
products. A diagnosis of pelvic peritonitis, parametritis and acute tubular necrosis was made
The course can be followed in the Figs 12 and 13
Penicillin therapy was continued to combat infection and she was placed on the high
calorie protein and mineral free dietary regime (7). This regime was continued for 15 days
and there was a gradual increase in urine output until on the 18th day after onset it exceeded
1 litre per day. During the oliguric phase her condition did not deteriorate despite the fact
that in this phase and in the early part of the early diuretic phase, mineral replacement was
withheld so as to demonstrate the tubular defect for conserving ions. She was as a result, in
strong negative sodium chloride and potassium balance and the plasma levels of these ions
fell. On the 27th day after onset, 1 litre of 5% glucose solution in excess of the calculated

requirement for the day was given intravenously and that evening she was stuporose. This stupor was reversed next day by the administration of sodium chloride and was probably the result of cellular overhydration. However, on the 29th day she again became confused, noisy, disorientated and developed a fine tremor of the hands with occasional twitching of large muscle groups. At this stage an intravenous injection of magnesium sulphate produced a transient cessation of the twitching and a type of phasic respiration. Later that night she had a fit and was sedated with sodium gardenal. A supplement of potassium chloride was added to the diet and from then on she improved gradually. By the 47th day when she was discharged from hospital she was almost back to normal health.
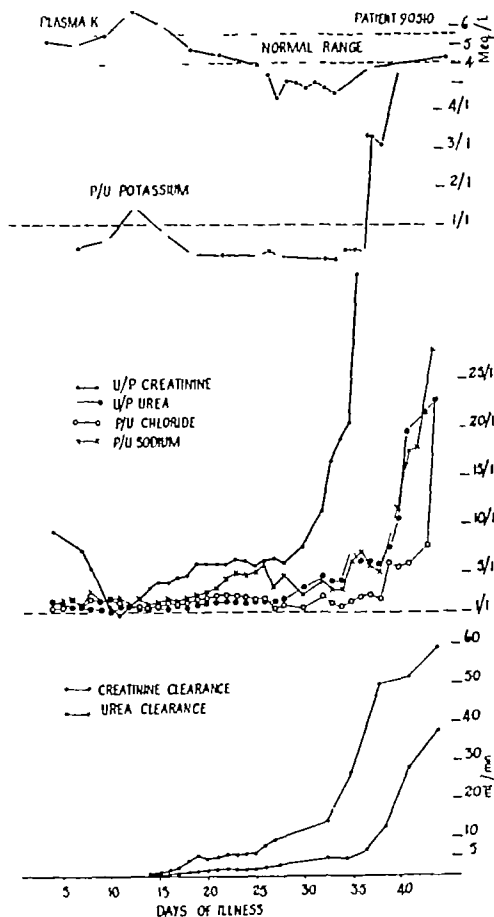

Fig 13

Conclusion. Acute tubular necrosis following abortion and probably due to a combination of shock and intravascular haemolysis (*cf* the jaundice). The early diuretic phase was complicated by disturbances in mineral and water balance.

Patient 91794, a man aged 47 years. On 30th Sept, 1948, he developed colicky abdominal pain, vomited brown and later bloody material and developed diarrhoea. The vomiting and diarrhoea increased in severity during the next two days. On 2nd October his wife noticed that he had passed no urine during the day. A doctor was called and failed to obtain any urine on catheterisation. Vomiting and diarrhoea continued, as did the anuria and he was admitted to another hospital on 6th October. There he was found to be hypertensive and to have a raised blood urea. The anuria persisted. On 8th October a laparotomy was performed and nothing abnormal found. A splanchnic block was carried out.

Renal functional data on patient 90510

Day after onset of anuria	44	84	191	102
Thiosulphate clearance, ml per min	50 66 5	100 70		
	Av 58 3	Av 85		
Para amino hippurate clearance, ml per min	132 173	310 266		447 492
	Av 153	Av 288		Av 470
Clearance thiosulphate ÷ Clearance P A H	38 4%	29 2%		
Urine specific gravity after 21 hours fluid deprivation			1028	

On 9th October he was transferred to Hammersmith Hospital. On examination he was a drowsy and unco operative patient. The blood pressure was 230/118 mm Hg. The state of hydration was normal with no œdema and no rise in venous pressure. The skin turgor and eyeball tension were normal. There was no pallor or jaundice and there was a recent laparotomy wound with generalised abdominal tenderness. For other details *see* Fig 14

    Blood urea   526 mg per 100 ml
    Plasma bicarbonate   8 1 m eq per litre
          „   potassium   6 6 m eq per litre
          „   calcium   3 2 m eq per litre
          „   phosphate   9 4 m eq per litre
          „   chloride   106 m eq per litre

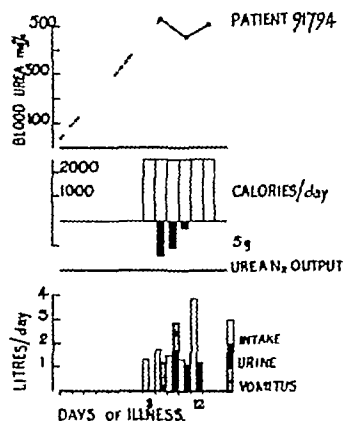On 10th October he showed evidence of left lower lobe pneumonia and parenteral penicillin administration was started

Fig 14

On 11th October he passed over 1 litre of urine. The urine urea concentration was very low with a urine/blood ratio of 1 78/1

On 12th October while coughing he burst his abdominal wound and this was repaired. His general condition deteriorated progressively. He lapsed into coma with jactitations before death on 14th October

Post mortem showed bilateral lobar pneumonic consolidation and acute tubular necrosis

Conclusion Acute tubular necrosis probably resulting from electrolyte and water loss due to diarrhœa and vomiting with consequent hæmoconcentration and gross compensatory reduction in renal blood flow Terminally this was complicated by bilateral pneumonia The hypertension on admission to Hammersmith Hospital was higher than seen in the acute renal failures in general but fell to lower levels before death

Patient 80753, a married woman aged 23 years, was admitted to Hammersmith Hospital on 7th February, 1949, following the onset of labour at the full term of her 2nd pregnancy She was delivered of a live child at 8 20 p m following a four hours labour However, she had a retained placenta with a continued moderate loss of blood so that at 9 35 p.m a transfusion of group O Rh negative blood was begun. Subsequently the placenta was manually removed under pentothal anæsthesia She was returned to the ward thereafter and transfusion was continued At 12 30 a m on 8th February, she developed a tonic spasm of the trunk and limbs with upward rotation of the eyes There was some frothy sputum at her lips The pulse rate was 80 per minute and blood pressure at this time 80/40 mm Hg The transfusion was immedi

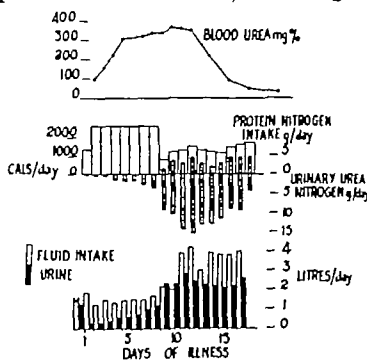Fig 15

ately stopped—about 1¼ pints had been given Within a few minutes her condition improved and the blood pressure rose to 110/70 mm Hg by 1 a m Later that day she passed bloody urine and was noticed to be slightly jaundiced As only a few ounces of urine were passed during the first half of 8th February despite good clinical condition and adequate fluid balance, she was allowed to come under our care Apart from some pallor and slight jaundice there were no abnormal physical signs and the patient felt well She was immediately placed on
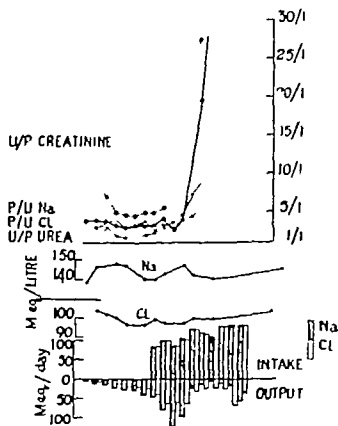

Fig 16

the conservative dietary regime (7) and continued on tube feeding for one week This was well tolerated and during this week there was a gradual increase in urine volume until she passed 1 litre on the 7th day Oral feeding of a low protein diet with salt supplements was begun on the 8th day Relevant data are shown in the adjoining Figs 15 and 16

Renal functional data on patient 80753

Day after onset of anuria	PAH clearance ml/min	Thiosulphate clearance ml/min	Creatinine clearance ml/min	Urea clearance ml/min	Cl thio / Cl PAH %	Extraction PAH %	Extraction creatinine %	Extraction thiosulphate %	RPF corrected
3	4.00	1.27	1.95	—	25.4	—	3.75	5.6	97.3*
10	11.5	5.08	—	—	48.2	8.45	—	5.41	120.3*
24	244	62.3	50.7	40.0	21.2	—	—	—	—

All clearances uncorrected for body surface area. To correct to area 1.73 sq metres multiply by 1.16

* Derived from clearances and extraction ratios — means

Uterine involution occurred satisfactorily The patient remained in good cond
throughout the illness despite the rapid rise in blood urea which was probably the resu
protein breakdown from the uterus The incompatibility of the blood transfusion was sh
to be due to a high titre of anti A body in the donor's plasma The patient belonged to grou
Functional data can be seen in the figures

Conclusion Acute tubular necrosis due to a rare type of incompatible blood transfusio

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DISTRIBUTION OF RADIOIODIDE IN MAN

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E E POCHIN *

(*From the Department of Clinical Research, University College Hospital
Medical School*)

WHEN radioactive iodine is administered to man in the form of iodide, it becomes distributed through the tissue fluids and is progressively removed from the plasma by the thyroid and kidneys   The course and extent of the thyroid and urinary accumulation have been repeatedly studied   The present paper deals primarily with the initial distribution of radioiodide throughout the body, since the speed and extent of this distribution, on which less information is available, influences the plasma radioiodide concentration and hence the rate of thyroid and renal removal of the dose given

Distribution of radioiodide through the body as a whole   After giving radioiodide intravenously, the amounts taken up by the thyroid and concentrated in the urine have been followed in the ensuing hours and expressed as percentages of the dose given, by methods previously described (4)   Since radioiodide is largely excreted in the urine (8), the amount in the extrathyroid tissues can be estimated from the difference between the dose given and the amounts present in thyroid and urine at any time   The data in Table I were obtained in 4 male and 8 female healthy medical students of average age 22, to whom 35 microcuries of carrier-free radioiodide had been given intravenously   Blood and urine samples were obtained after 1, 2, 4 and 6 hours, thyroid and thigh counts being made at repeated intervals during this period   At six hours the average thyroid and urinary contents were 25 and 39% respectively, so that the remaining tissue content had fallen to 36% of the dose

If this extrathyroid tissue content is compared with the simultaneous value of the plasma concentration, the extent of the radioiodide space may be calculated   The mean plasma concentration at 6 hours was 1 4% of the dose per litre, so that a volume of about 25 litres would be required to accommodate the observed tissue content at a concentration equal to that in plasma   As with other conventional diffusion spaces, it is not assumed that an anatomical volume of this extent is filled with fluid at uniform concentration

The volume so calculated will apply to iodide until appreciable amounts of radioiodine are discharged from the thyroid into the plasma in organic combination   We have used a modification of Taurog and Chaikoff's butanol extraction (3) to detect the presence of radioactive thyroxine   In normal subjects, radioiodine has been detected in the thyroxine fraction at 48 hours but not at 24 hours from the dose   In patients with Graves's disease, it has been observed at 3 hours in 2 subjects and repeatedly at 7 hours   The volume calculated should therefore measure the radioiodide space at least for the first 6 hours in normal subjects and for the first hour in thyrotoxic patients   Its value becomes uncertain after these periods in any case, since most of the dose is then in thyroid or urine, and the tissue content, as estimated by difference, is greatly influenced by small errors in calibration of thyroid or urinary content
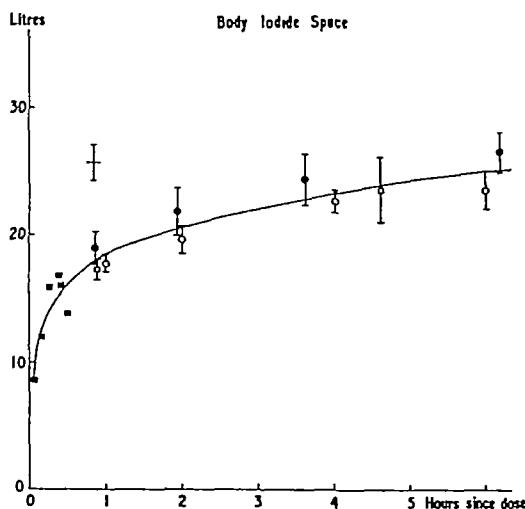

Fig 1   Volume of radioiodide space in litres   open circles, normal subjects after I V dose , open squares, euthyroid patients after I V dose , black squares, individual patients after I V dose   black circles, patients after oral dose , crosses, patients with Graves's disease Vertical ranges are those included by ± 2 Standard Errors

In Fig 1, the mean radioiodide space so calculated for the normal subjects is seen to increase from 18 litres at 1 hour to 25 litres at 6 hours Similar values have been obtained in patients without thyroid disease, for whom individual values at periods shorter than 1 hour after an intravenous dose are also plotted   Radioiodide therefore becomes distributed in normal subjects through a space which increases rapidly during the first hour, and at a slow and probably decreasing rate in the ensuing 5 hours, during which reliable values may still be obtained

In Graves's disease, values should be reliable for the first hour at least, and at this time have been found to be higher than in subjects without thyroid disease (Fig 1), as Keating and Albert have also found (1)   A small

component of the body iodide space will be omitted from this estimate, since radioiodide in a constant volume of extrathyroid neck tissues in sight of the counter will influence the neck count, and will be included as thyroid content if no correction is made   An approximate correction may be applied to the neck counting rate to separate the effect of neck tissues from that of the thyroid, by subtracting from it the counting rate observed opposite the thigh   In cases of myxœdema, or in normal subjects within a few minutes of an intravenous dose and before appreciable thyroid accumulation has occurred, it is found that the neck count is about equal to the thigh count, with the positions and shielding used in this work   When the results are corrected in this way, the body iodide space is increased by an average of 1 2 litres which agrees well with the volume calculated to be in sight of the counter

Distribution of radioiodide through thigh tissues   If a suitably shielded counter is placed in a constant position relative to the thigh, the counting rate should be proportional to the amount of radioiodine within a constant volume of thigh tissue, namely that from which radiations are detected by the counter   If, therefore, this thigh counting rate is compared with the simultaneous value of the plasma radioiodide, the ratio should give a measure of the volume of plasma, or fluid of equal radioactivity, contained in a sample of limb tissue of constant volume   It is assumed that in such a structure as the thigh, the counting rate will not be influenced appreciably by changes in the mean position of radioiodine atoms within the thigh, but only by their number

When a series of such measurements is made in a subject after an intravenous dose of radioiodine, the ratio of thigh count to plasma activity is found to increase progressively, indicating an increasing entry of radioiodine into the extravascular thigh tissues   It therefore becomes useful to express this value in absolute terms, for example, as the percentage of the thigh volume occupied by iodide in a concentration equal to that of the plasma

This percentage cannot be measured directly since the exact volume and position of tissues ' in view of the counter " is difficult to determine   A rough estimate can be made, however, of the count that would be observed if the whole thigh volume were occupied by plasma, and the following experiment was designed to simulate this condition   Long cylindrical glass vessels of different diameters were filled with water containing radioiodide at equal concentration in each vessel   The counter was then placed alongside each vessel with the axes of counter and vessel parallel and the counter shield (4, Fig 1) touching the vessel wall   This relative position corresponds to that used in making thigh counts when the counter shield is placed against the skin of the thigh   In these circumstances, the counting rate for different vessels increased with increasing vessel diameter (Fig 2)   It will be observed that for vessels of the size of a normal thigh, the counting rate does not vary greatly with the circumference of the vessel, a 10% increase in circumference being associated with a 5% increase in counting rate   This model will only crudely simulate conditions in a tapering thigh with a centrally placed femur, and where the ' avascular " boundary is a skin layer rather than a thicker glass wall   It may, however, allow a rough comparison to be made between thighs of different size, and give an approximate estimate of the proportion of thigh tissue through which diffusion of iodide has occurred   In practice, the thigh count in a standard position is related to the size of the dose given , and the simultaneous plasma concentration is expressed as a percentage of the dose per litre   From the data of Fig 2, a " thigh count " may then be estimated corresponding

to a given dose, thigh size and plasma concentration, and to complete penetration of radioiodide throughout the thigh   Comparison between observed and calculated values estimates the percentage penetration at the time of observation

Thigh counts were made with the leg resting horizontally on a lead sheet and placed between a vertical lead sheet 16 cm high by 36 cm wide and 1 3 cm thick, and a G 10 counter   The counter axis was parallel to the leg and 6 cm from its lateral surface, and the counter centre was opposite a point 15 cm above the upper border of the patella, the knee being flexed

Normal diffusion of iodide through the thigh, as studied in this way, is found to progress rapidly during the first hour after an intravenous dose, and then more slowly for several hours (Fig 3)   The extent of the diffusion was evaluated by this method in 12 normal subjects (Table I) and 8 patients at one hour after the dose, and then averaged 35% of the thigh volume
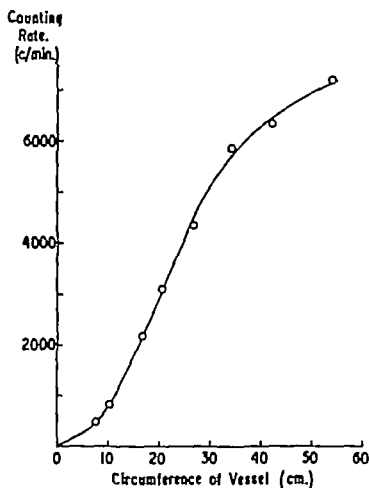

Fig 2   Variation of counting rate with circumference of cylindrical glass vessels , counter at constant distance from wall, and vessels all containing fluid of equal radioiodine concen tration

As in the whole body, the iodide diffusion volume of the thigh was greater in thyrotoxic than in normal subjects   We have no evidence whether this represents a more rapid attainment of the same diffusion equilibrium or whether the final iodide space is increased in thyrotoxicosis

The diffusion of iodide, as examined in the thigh, clearly does not give an accurate sample of diffusion through the body as a whole   It may, however, indicate diffusion through the greater part of the body tissues and therefore give a close estimate of such diffusion   It has, moreover, the advantage that it is not dependent on a difference between measurements, and is not sensitive to the errors which vitiate the whole-body determinations within a few hours of the dose   It may, therefore, be continued for several days provided that accurate plasma and thigh counts are obtainable

Diffusion of radioiodide into erythrocytes   When normal blood is mixed with radioiodide solution in vitro and is centrifuged after 3 minutes of mixing, the partition of radioiodine between cells and plasma is the same

as after 8 hours equilibration   In consequence of this rapid diffusion into red cells, measurements may be made on plasma separated immediately from freshly drawn blood, without error caused by recent passage of part of the blood through the thyroid gland

The radioiodide concentration in packed cells after such mixing in vitro has averaged 0 65 times that of the corresponding plasma   This ratio agrees with that for blood from patients to whom radioiodide had been
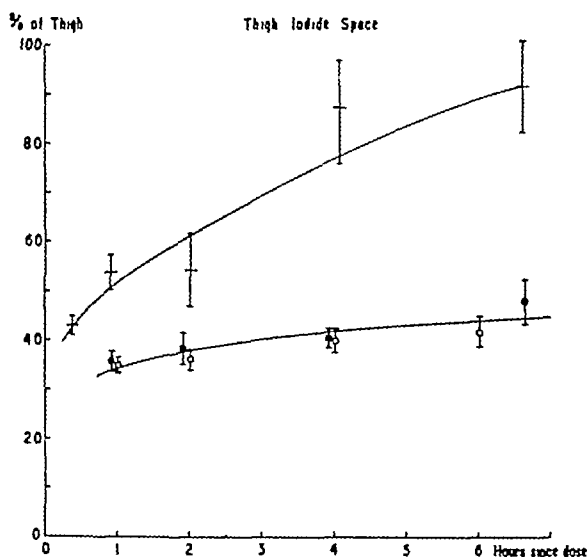

Fig 3   Estimated radioiodide space of thigh tissues   open circles, normal subjects, black circles, euthyroid patients, crosses patients with Graves's disease   Vertical ranges are those included by ± Standard Errors

administered in the preceding hours, and in whom butanol fractionation showed the radioiodine to be present as iodide   The mean ratio in normal subjects 1 hour after an intravenous dose has been 0 68 (Table I)

The true concentration ratio is likely to be lower by a few per cent owing to plasma contained in the red cell mass, which was spun off for 10 minutes at 3,000 r p m in wide bore tubes, and then lysed in 0 03% saponin solution and counted in a liquid counter

Concentration of radioiodide in alimentary secretions   Schiff and others (7) have shown that after intravenous administration, radioiodide becomes highly concentrated in gastric juice and in saliva   In the mixed oral saliva of the 12 normal subjects (Table I), we have found radioiodide concentration to average over 30 times those of the plasma samples taken simultaneously at intervals from 1 to 6 hours after an intravenous dose of carrier-free radioiodide   When average concentrations are plotted (Fig 4), the salivary

TABLE I

Results in normal subjects

Sex	Age	Radioiodide at 1 hour in					Radioiodide space at 1 hour in			Clearances	
		Urine %	Thyroid %	Plasma %/l	R B C %/l	Saliva %/l	Whole body		Thigh %	Thyroid ml/min	Renal ml/min
							Litres	% b wt			
F	19	10 1	5 5	4 7	3 5	330	18	24	28	20	30
F	20	14 1	4 0	4 0	2 8	160	20	27	40	14	32
F	20	9 3	6 6	5 4	3 8	430	16	25	31	9	33
F	20	14 5	8 3	5 6	3 5	300	14	23	25	15	20
M	20	0 6	12 5	3 5	2 0	230	22	36	40	20	21
M	22	12 5	12 1	3 6	2 7	60	21	34	42	37	47
M	24	0 8	13 3	4 1	2 8	130	10	24	38	32	24
F	23	13 2	7 3	3 8	3 4	110	21	28	38	28	40
F	25	14 3	10 0	4 3	2 9	240	18	26	37	25	42
F	29	11 1	14 4	3 8	2 4	310	20	20	30	33	43
F	20	21 4	12 6	3 4	2 2	170	19	27	30	30	53
F	20	13 3	10 0	3 6	2 2	200	24	32	35	28	45
Mean	22	12 4	9 7	4 2	2 8	230	18	28	35	25	36
S E		1 0	1 0	0 2	0 2	27	0 8	1 2	1 5	2 4	2 9
Coeff of var %		29	33	17	21	40	14	15	15	34	28

concentration is seen to fall on a curve parallel to that for plasma concentrations, and displaced from it along the time axis by about half an hour The data of Fig 4, where salivary concentrations are plotted on one-thirtieth the scale of that for plasma, are consistent with the hypothesis that saliva collected in the mouth contains radioiodide at 30 times the concentration of that in plasma 30 minutes previously In gastric juice collected by aspiration, the average concentration ratio relative to plasma in 3 patients was 40, high values having been recorded in one case of histamine-fast achlorhydria and in one subject in whom the swallowing of saliva was
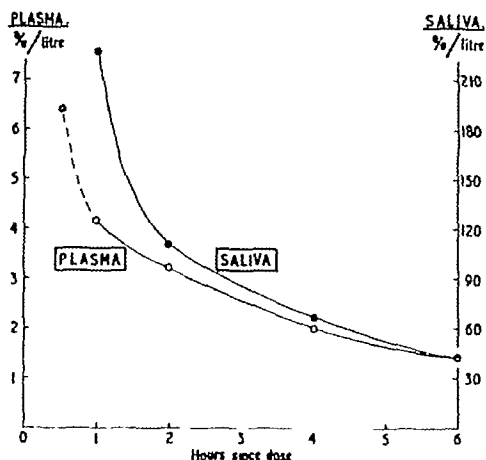

Fig 4 Radioiodine concentration in plasma (open circles) and saliva (black circles) after I V dose in 12 normal subjects (The plasma value at ¼ hour is derived from a separate group of normal subjects) Scale for saliva 1/30 that for plasma

excluded A fall of radioiodide concentration parallel to that in plasma was observed in gastric juice as in saliva

It is of some interest to notice the type of effect that gastric and salivary secretion of radioiodide might have on the estimated radioiodide space If, for example, 10% of the dose were passed in this way into the stomach, at a time when plasma concentration was 5% per litre, the gastric contents would be represented by 2 litres of the estimated radioiodide space Suppose now that this material, containing 10% of the dose, was still passing down the alimentary tract at a time when the plasma concentration had fallen to 2% of the dose per litre It would then account for 5 litres of the radioiodine space, and it is seen that the value of this space as normally calculated may increase owing to radioiodine remaining in the gut while the plasma concentration falls, and without any further diffusion of radioiodine taking place A similar progressive rise in the value obtained for the space would clearly occur also if the processes of gastric excretion and later re absorption of radioiodine were continuous The progressive increase in the radio iodide space after one hour may be due in part to this effect, but the corresponding rise in thigh space shows that other factors are also involved

It will also be noticed that, the faster the plasma concentration falls, the greater will be the rise in the estimated radioiodide space due to this effect The increased space in Graves s disease may be partly due to the more rapid fall of plasma concentration in these patients Their increased thigh space shows that other factors are again involved

Diffusion of radioiodide into other tissue fluids   A few observations have been made on the entry of radioiodide into œdema and ascitic fluid Radioiodide was injected intravenously into a patient in congestive cardiac failure, from whose legs œdema fluid was draining by Southey's tubes at a rate of 30 ml per hour   A series of samples of plasma and of the issuing œdema fluid were obtained in the following 6 hours on two occasions   The results (Fig 5) show that the radioiodide concentration in œdema fluid obtained under these circumstances approaches that of plasma in the course of 5 hours, and then falls as the plasma concentration falls   The œdema fluid concentration had approached to within 75% of the plasma concentration in 3 5 and 4 0 hours in the two investigations that have been made
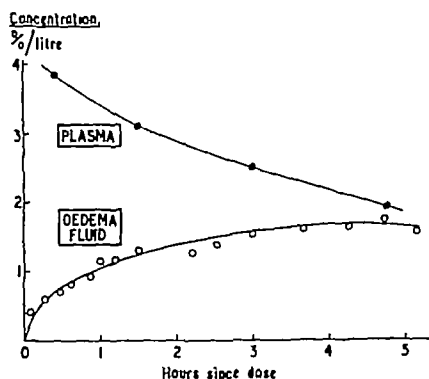

Fig 5   Radioiodine concentration in plasma (black circles) and œdema fluid draining by Southey's tubes (open circles) after an intravenous dose in a euthyroid cardiac patient

Similar investigations have been made by injecting radioiodide intravenously during removal of ascitic fluid in two subjects   The radioiodide concentration in the ascitic fluid again approached that of plasma, reaching 75% of its value after 0 5 and 3 3 hours in the two cases (Fig 6)   In both these instances, part of the delay in equilibration between plasma and fluid must have been due to diffusion of radioiodide from capillaries into the bulk of the fluid concerned, which must in general lie at a much greater average distance from the nearest capillary than under physiological conditions   It is of interest, however, that the same rather slow equilibration is observed, as was seen in the case of the radioiodide space in the body as a whole and in the thigh

DISCUSSION

After radioiodide has been given intravenously, the plasma concentration falls progressively as it is removed into the thyroid, the urine, or the tissue radioiodide space   The thyroid and renal removal differs from that into the tissues, however, in two ways   Firstly, both thyroid and kidneys clear a constant volume of plasma of its radioiodide each minute, so that their rates of uptake are proportional to the plasma concentration , whereas the

loss to the tissues proceeds at a decreasing rate as equilibrium is approached Secondly, the radioiodide removed by thyroid and kidneys is withdrawn from communication with the plasma   That in the tissue space is assumed to remain in communication with plasma so that its amount falls as the plasma concentration is lowered by thyroid and renal action, although radioiodide in the alimentary secretions may differ by not remaining continuously in such communication

Knowing the extent of the radioiodide space, we can define the relationship between thyroid uptake and the filling of this space , and can analyse the factors which influence the plasma radioiodide concentration
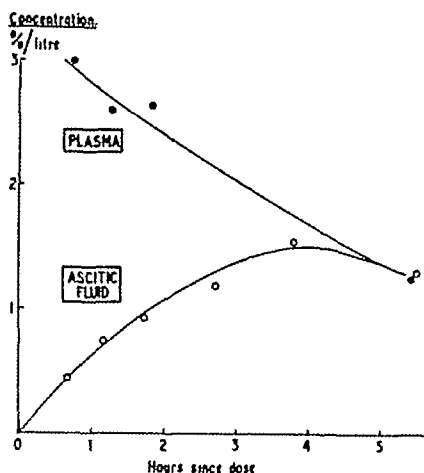

Fig 6   Radioiodine concentration in plasma (black circles) and ascitic fluid (open circles) after an I V dose to a patient with portal obstruction

It can be shown (4) that this plasma concentration falls with time according to the relation

$$-\frac{1}{c}\frac{dc}{dt} = \frac{g + r}{V} + \frac{1}{V}\frac{dV}{dt} \tag{1}$$

where plasma concentration (c), radioiodide space (V) and time (t) are variable, while thyroid (g) and renal (r) clearances are constant   This formula asserts that, over any short time interval, the percentage fall of plasma concentration is accounted for by the percentage of the current radioiodide space cleared by thyroid and kidneys, and the percentage increase in the radioiodide space itself

The relative importance of the factors lowering plasma concentration in normal subjects is seen from Fig 7   Taking average normal thyroid and renal clearances at 25 and 32 ml /min , (g + r) is constant at 57 ml /min

The rate of increase of the radioiodide space $\left(\dfrac{dV}{dt}\right)$ however, as measured

from the slope of the mean curve in Fig 1, falls rapidly in the first hour after an intravenous dose and then more slowly in the ensuing hours, having a value of 50 ml /min at 1 hour and a less accurately determined value of about 15 ml /min at 4 hours   It is, however, clear that escape of radioiodide into the tissues will largely determine the fall of plasma concentration, and hence greatly influence the thyroid and renal uptake rates, during the first hour   For several hours it will continue to be of greater importance than the rate of thyroid clearance   This and other consequences of the distribution of radioiodide affect certain indirect tests of thyroid activity that have been proposed or used clinically   It may, therefore, be useful to review the factors upon which such tests depend and their variability

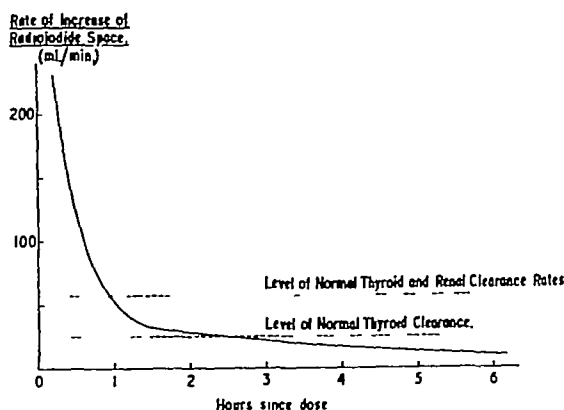

Fig 7   Rate of increase of radioiodide space in euthyroid subjects derived from Fig 1   The effect of this increase in lowering plasma concentration equals that of the normal thyroid after 2½ hours

Test based on analysis of excretion rates   Keating and others (2) have suggested that, when radioiodide reaches diffusion equilibrium through the tissues, the fall in plasma concentration is due to thyroid and renal removal only and, if the latter is known by urinary measurements, the former may be inferred   It is clear that such an analysis cannot safely be started within 6 hours of the dose in normal subjects, since otherwise the continuing slow expansion of the radioiodide space will be reckoned as thyroid uptake   And it is likely that, if later urine samples only are used, the fitting of an exponential and linear formula may involve appreciable uncertainty in many cases   In Graves's disease, although the radioiodide space is greater, it is likely that valid results would be obtained from an hour after an intravenous dose, in view of the considerable increase of the thyroid clearance rate

The delay in attaining equilibrium in the tissue distribution of radio-iodide also affects the relevance of Oddie's mathematical analysis (5) of radioiodine distribution   His proposals assumed distribution equilibrium

at zero time and so only operate from initial conditions at which the thyroid and urine have high radioiodine contents, and are not applicable to much of the uptake phase

Evidence from total urinary excretion Since the thyroid and the kidneys each clear a roughly constant volume of plasma of its radioiodide each minute, the thyroid and renal uptake rates should always be in proportion to the ratio of these clearance rates Moreover, since thyroid uptake is in most cases largely complete before appreciable radioiodine is discharged from the thyroid, the radioiodide contents of the thyroid and of the total urine should be in about the same ratio It was found that, at 24 hours from an oral or intravenous dose, the percentage of the dose in the thyroid gland (G) and that in the urine (R) were related to the thyroid (g) and renal (r) clearances by the formula

$$\frac{g}{r} = K \frac{G}{R} \qquad (2)$$

where K had a mean value of 0 95 but varied with a standard deviation of 0 23 If R alone is measured, as in the normal clinical test based on urinary excretion of a test dose, certain inferences may be made as to the value of g, since

$$g = Kr \frac{G}{R} = \frac{Kr}{R} (100 - R - B) \qquad (3)$$

where B is the percentage of the dose present in the extrathyroid tissues at 24 hours

The thyroid clearance will correlate highly with the urinary output if K, B and r vary little in different subjects It is found that the greatest contribution to the total variance (Table II) is due to individual variability of the renal clearance for iodide which, in 49 subjects without evidence of renal disease, has had a mean of 32 1 and a standard deviation of 12 0 ml /min

TABLE II

Parameter	Mean value	Coeff of Var %	Contribution to total variance %
K	0 95	23	24
100 − R − B	80% − R	17 (at R = 50%)	13
r	32 ml /min	37	63

If the values of Table II are inserted, equation (3) becomes

$$g = \frac{30}{R}(89 - R)(1 \pm 0 47) \qquad (4)$$

and it has been shown (6) that this curve and the associated limits correspond

to the observed values when 24 hour urinary output is plotted against thyroid clearance The known sources of uncertainty in the urinary excretion test could thus account for the observed variability of its results and for overlap between values in thyrotoxic and normal subjects In a few thyrotoxic subjects, the relationship fails if by 24 hours any considerable output of radioiodine from the thyroid has occurred, when the extrathyroid radioiodine *B* may be great and the data from two such subjects have been excluded from the analysis of Table II

Inferences as to thyroid uptake which are based solely on urinary output, therefore, involve an error (S D) of at least 47% due to uncontrolled variables, of which the renal clearance is the most important except in cases with rapid thyroid output of labelled hormone In tests of thyroid function
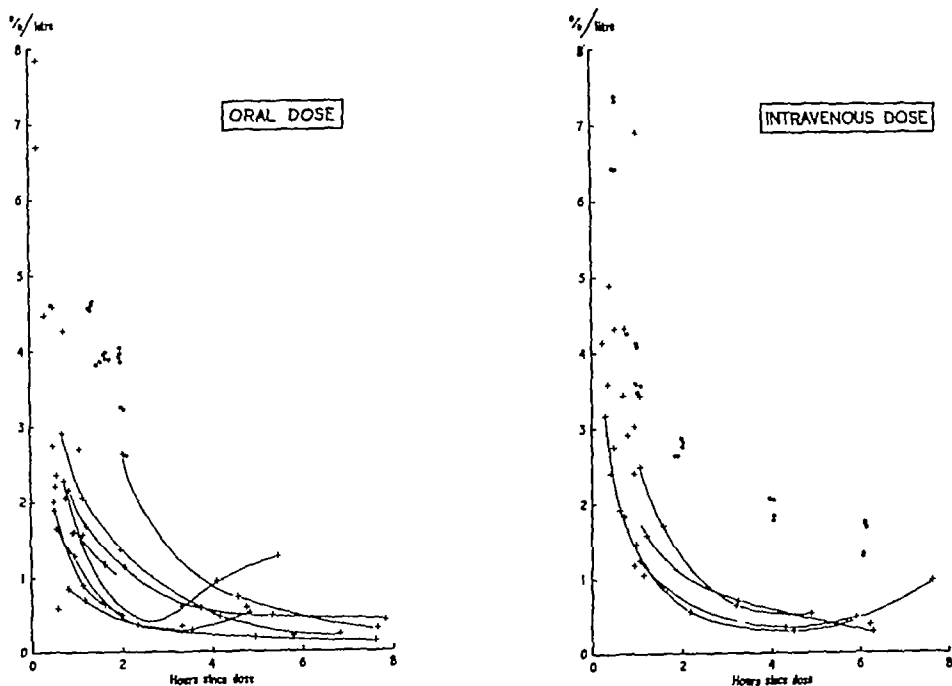

Fig 8   Radioiodine concentration in plasma after (*a*) oral and (*b*) I V dosage in euthyroid subjects (black circles) and in patients with Graves s disease (crosses)

based on urinary output over different time periods, the influence of these factors will vary The variability of renal clearance must always impose an error of 37% (S D) which could be avoided by assessing thyroid function instead from the plasma radioiodide concentration during the corresponding interval

Tests based on plasma concentration   The rapid depletion of plasma radioiodide by an overactive thyroid is evident from the data of Fig 8 For the first hour, the ranges of plasma concentration in normal and

thyrotoxic subjects overlap, presumably since at this time the expansion of the radioiodide space is the main factor influencing plasma concentration in both groups   After 2 hours, values in the thyrotoxic subjects studied have fallen below the normal range except in one subject whose oral dose was known to be slowly absorbed from the stomach   After 4 hours, normal values are also falling to a low figure and, if the plasma value is based simply on total radioiodine and not on radioiodide alone, the concentrations in some thyrotoxic subjects are rising owing to release of radiothyroxine   It seems likely, therefore, that a useful diagnostic test of untreated Graves's disease could be based on the plasma radioiodine concentration at 2 hours after an intravenous dose

The rate of fall of plasma radioiodide concentration later than 12 hours after the dose indicates the total clearance by thyroid and kidney, as is seen by equating $\dfrac{dV}{dt}$ to zero in equation (1)   This, or the parallel fall in renal excretion rate, may afford a useful index of myxoedema, as Skanse (8) has shown, but its value will always be disturbed by variability of the renal clearance

Tests based on thyroid content   The maximum thyroid content after a test dose of radioiodide, or the content at 24 hours, have little advantage over the corresponding urinary outputs as a test of thyroid activity   Since at this time most of the dose is usually shared between thyroid and urine, both measures give almost the same information and have the same sources of variability   In hypothyroidism the thyroid activity is, however, more accurately assessed by counts over the thyroid at this period than from total urinary output

The rate with which the thyroid accumulates radioiodide in the first hour after a dose, particularly if given intravenously, gives a much better index of thyroid activity, since when calibrated in terms of the dose given it depends on thyroid clearance and plasma concentrations only   And it has been shown that the average plasma concentration does not differ greatly in normal and thyrotoxic subjects during the first hour   It therefore gives information which is inferior to the thyroid clearance only in ignoring the difference in plasma concentration

The test may be improved in this respect if the count opposite the thigh is used as a measure of plasma concentration, and this is valid in so far as the " thigh space " is equal in different subjects at this time   The more active the thyroid in taking up radioiodide, the greater the neck count becomes at 1 hour after the dose, and the less radioiodide remains in the rest of the body as judged by the thigh count   The ratio of neck count to thigh count proves therefore to be a sensitive index of thyroid uptake, correlating closely (r = 0 94) with the thyroid clearance (6)   This ratio rises linearly with time in normal subjects from an initial value of about

unity   The test has the clinical advantages that it is rapidly made, it requires only 10 microcuries or less of radioiodide, and this dose need not be accurately standardised, since the result depends upon a ratio of counting rates   Errors due to delay in gastric absorption are unusual if the dose is given orally at 3 hours after a light breakfast, but intravenous administration can be used to avoid this error

Thyroid clearance determinations   The tests already discussed have been indirect estimates of the aspect of thyroid function measured directly by the thyroid clearance   They must therefore all involve uncertainty from uncontrolled factors not affecting the thyroid clearance determination, which is based directly on thyroid uptake at a given plasma concentration The thyroid clearance has been found to be more reliably and conveniently estimated by intravenous than by oral dosage, since the radioiodine content of general neck tissues is then more constant and the dose may be given at any time of day regardless of gastric content   We have corrected for the neck content in the manner described above by subtracting the value of the thigh count from that opposite the neck in order to estimate the activity due to the thyroid gland itself   The thyroid clearance of 12 normal subjects averaged 25 ml /min (with S D 8 4 and S E 2 4), values in subjects without thyroid disease ranging from 7 to 42 ml /min , while the intravenous clearances of 12 subjects judged to have Graves's disease ranged from 84 to 350 ml per minute   The occasional high values of over 400 ml per minute observed by the oral method (4) have not been found, but since the cases studied by intravenous dosage have been more recent and investigated more for diagnostic purposes in doubtful cases than as a representative survey of Graves's disease, it is likely that the average value would be lower, and values of oral and intravenous clearances have agreed well in the few subjects in whom both have been determined   In the whole group studied, the thyroid clearance has been raised from a normal value of 25 ml per minute to an average value of 240 ml per minute in 21 cases of Graves's disease, with some selection of mild cases in this group   It is clear that when the thyroid clearance is raised tenfold in the average case, all indirect tests of thyroid uptake are likely to be clearly positive   When, however, the thyroid clearance is raised only from 25 to 100 ml per minute in mild cases, it is likely that certain indirect tests may give ambiguous results, for example, if the raised thyroid clearance is associated with a high normal renal clearance so that the sharing of the dose between thyroid and kidneys lies within the normal range   In such cases, the thyroid clearance itself, or some other and simpler test highly correlated with it, may be used in the diagnosis of a raised iodide uptake by the thyroid

SUMMARY

1   After carrier-free intravenous injection in man, radioiodide becomes distributed through 18 litres or 28% of the body weight in one hour and through a slowly increasing volume in the following five hours

2 The penetration into thigh tissues, and into cardiac œdema fluid and ascites, is not completed within several hours, whereas that into erythrocytes is rapid

3 In saliva and in gastric juice, the radioiodide concentration exceeds thirty times that in plasma

4 Indirect tests of thyroid iodide uptake may be related to the thyroid clearance rate and the sources of variability in certain such tests are defined

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THE METABOLISM OF RADIOTHYROXINE IN MAN

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LEBLOND (8) has shown that when small doses of labelled thyroxine are injected into rats, radiothyroxine is selectively concentrated in the liver and secreted into the bile, and radioiodide is excreted in the urine   Since the doses used were too small to influence appreciably the total amount of endogenous hormone, these results may be taken to indicate the behaviour of the natural circulating thyroxine   Similar work on normal men has not been published, but Keating and Albert have reported results following the oral administration of 1 mg doses of labelled thyroxine to 3 subjects with myxœdema (5)   They found that thyroxine was absorbed more slowly than iodide, and that about 25% of the I^{131} in the dose was excreted in the urine as iodide by the end of 24 hours

We have studied the fate of physiological doses of labelled thyroxine given intravenously and by mouth to normal humans   We have also compared the metabolism of radiothyroxine obtained by artificial synthesis and by natural synthesis in the thyroid glands of subjects given I^{131} as iodide

Materials and Methods

Radioactive thyroxine   The labelled thyroxine was prepared by the iodination of 3 5 diiodothyronine with carrier-free radioiodide   The product of this reaction is shown at Fig 1
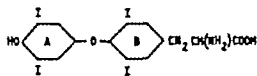

Fig 1   Radioactive thyroxine labelled in 3 and 5′ positions

The thyroxine was made up in $M/50$ Na_2CO_3 as the monosodium salt Each dose contained 80 micrograms of carrier thyroxine, and the activity of the doses ranged from 15 to 25 microcuries   In two of the eight doses given by injection *l*-thyroxine was used   In the other six, and in the two given by mouth, *dl*-thyroxine was used

Estimation of radioactivity   The proportion of the dose in the thyroid was measured with an externally placed gamma-ray counter, using a method previously described (10)   The radioactivity present in body fluids was measured with a thin-walled glass counter of capacity 10 ml , giving a response of about 10,000 counts/minute for one microcurie   Radioactivity in the red cells was measured in 10 ml samples made from a known volume of cells hæmolysed by saponin   Radioactivity in the stools was measured with a gamma-ray counter at a standard distance

Analysis of samples   Identification of the chemical form of the radioiodine was not always possible, but in some of the fluid samples we determined roughly the proportions in the iodide and thyroxine fractions by a modification of the method of Taurog and Chaikoff   The plasma iodine was taken up by shaking with 5 volumes of butyl alcohol, and the inorganic iodine removed from the butyl alcohol by 3 separate extractions with alkali

The proportion in the iodide and thyroxine fractions in two of the samples used for making up doses was estimated by paper chromatography   In each analysis, 0 008 ml containing added thyroxine and iodide carrier were run on Whatman No 4 filter paper strips, using water and phenol as solvents   In each strip, two separate areas of radioactivity were defined by their blackening effect on X-Ray film plates, and were shown to correspond with areas containing the iodide and thyroxine identified chemically   The radioactive material from each area was washed off the paper and measured in the liquid counter   In one sample, from which two of the doses were made up, 11% of the radioactivity was due to iodide   In the other sample, from which three doses were taken, iodide accounted for 9% of the total *

This method could not be used in the analysis of plasma and urine samples owing to their low activity

Procedure   As soon as the dose was given, measurements were made over the neck and thigh, and were repeated at frequent intervals for the first six hours   Urine was collected at intervals of about half an hour for the first six hours, and then every few hours until the end of the experiment   Blood samples, each of 20 ml , were taken at 15 minutes, and at 1, 2, 4 and 6 hours   At the end of the 24-hour period, a blood sample was taken and final measurements made over the neck and thigh   In a few cases measurements were continued daily until the third day

Results of intravenous injection

Measurements were obtained on the thyroid, blood and urine in 6 euthyroid people   In a seventh subject, plasma and urine samples only were measured   Since there was no systematic difference between the

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* We are grateful to Dr J C Laidlaw for carrying out one of these analyses

results with *dl-* and *l-*thyroxine (Tables II and III), the two experiments with *l-*thyroxine are not described separately

The change in plasma concentration   Fig 2 shows the change in total plasma radioactivity during the first 24 hours   Two phases in the rate of fall may be distinguished in the plasma radiothyroxine curves   At first, the concentration falls rapidly to a level of about 5% of the dose per litre at the sixth hour   Then there is a slower fall to 2 or 3% by the 24th hour In the subjects on whom measurements were taken on the second and third days, this concentration was maintained
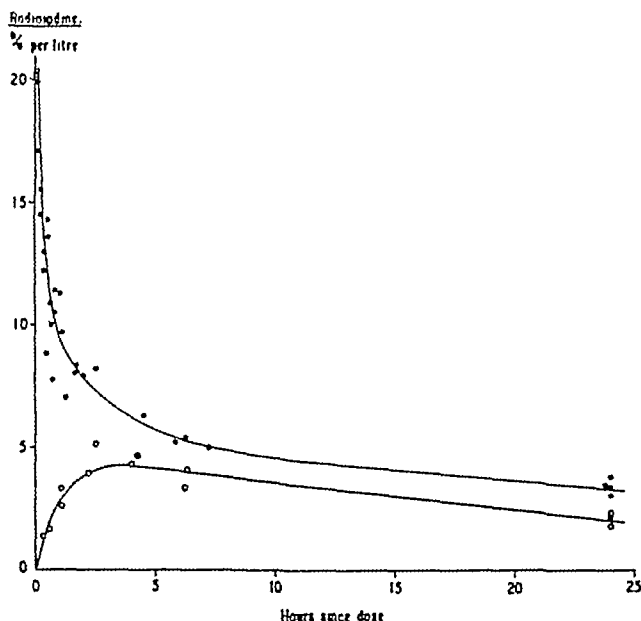

Fig 2   Plasma radioiodine concentration   Closed circles after intravenous sodium radio thyroxine , open circles after oral sodium radiothyroxine

The proportion of the total plasma radioiodine due to thyroxine cannot be determined accurately by the chemical method, because of low recovery in the butyl alcohol extract   About 28% of the total radioactivity remains behind in the plasma [*]   However, in samples analysed at various time intervals between the 15th minute and 6th hour in 3 cases, we found that 90% or more of the extractable radioiodine was due to thyroxine, the remainder of this part being due to iodide   There was no systematic difference between the values from the early and late samples   An indirect, but probably more accurate, estimate of the proportion of iodide in the total

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[*] This low recovery has been obtained consistently when radioiodide is added to plasma in vitro, and is probably due to adsorption of iodide on to the protein precipitated at the butyl alcohol water interface

plasma radioiodine can be made from the excretion rate of radioiodide in the urine, and the renal iodide clearance rate. Values calculated on the assumption that the renal clearance was 32 ml /min (10) are given in Table I. In the calculations of thyroxine distribution discussed below, we assume that 90% of the plasma radioiodine is thyroxine, and the remainder iodide.

TABLE I

Plasma radioiodide calculated from urinary excretion rate and renal clearance rate of iodide assumed to be 32 ml /minute Values expressed as % of total radioiodine in plasma (last column in each period)

Subject	1 hour			6 hours			24 hours		
	Plasma radioiodine			Plasma radioiodine			Plasma radioiodine		
	Total %/l	As iodide %/l	% as iodide	Total %/l	As iodide %/l	% as iodide	Total %/l	As iodide %/l	% as iodide
1	9 4	0 1	1 1	7 0	0 4	5 7	3 8	0 4	10 5
2	7 3	1 1	15 1	3 5	0 3	8 6	2 0	0 3	15 0
3	10 1	1 0	9 9	5 4	0 7	12 9	3 6	0 4	11 1
4*	10 0	1 2	12 0	5 1	0 7	13 4	3 4	0 4	12 1
5*	10 8	0 9	8 4	5 7	0 4	7 0	3 3	0 3	9 1
6	9 0	0 6	6 7	4 5	0 2	4 5	3 4	0 2	5 9
Average			8 9			8 7			10 6

* *l* thyroxine used

Accumulation in the thyroid Fig 3 shows a typical thyroid uptake curve, obtained by external counting at the neck. Up to the first hour the
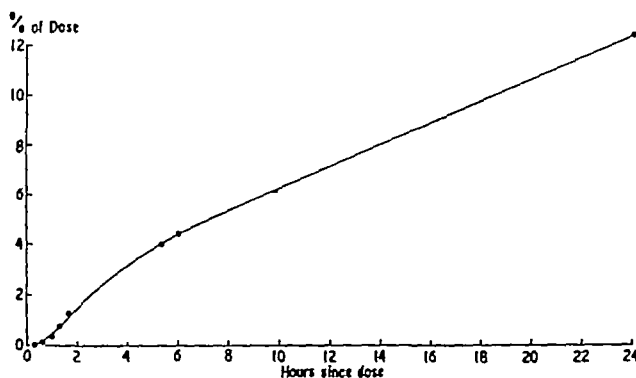

Fig 3 Uptake of radioiodine by the thyroid after injection of sodium radiothyroxine (subject 4)

amount of radioactivity in the thyroid was *too small to be discriminated* with certainty from that present in neck muscles, but thereafter there was

a slow and roughly constant rate of accumulation   This cannot be due to the thyroid clearing radiothyroxine from the plasma, since the rate of uptake of radioiodine is independent of the plasma concentration   If the radio-activity detectable in the thyroid were due to uptake of I^{131} as radiothyroxine, the rate of increase should be high initially, and should fall rapidly as the plasma concentration falls during the first five hours   Since radioiodide was present in the earliest urine samples obtained, we may assume that accumulation of iodide also is responsible for the radioactivity in the thyroid   This assumption was tested further in one case   The total radioiodine in the thyroid and the radioiodide in the urine were measured over a standard period from the time of injection, and the ratio of the two quantities calculated   This ratio was also calculated after a dose of radioiodide had been given several weeks later, and was found to be the same (42% going to the thyroid in each case) as the ratio obtained when radiothyroxine was injected   It follows, therefore, that the thyroid and kidneys have the same relative avidity for iodide and the iodine accumulating in the thyroid after radiothyroxine injection

Further evidence was obtained in a single patient with Graves's disease in whom we measured thyroid and urine accumulation after an intravenous injection of radiothyroxine   In this case the thyroid uptake of radioiodine was 4 2 times the urinary excretion, although the sum of the totals in the thyroid and urine (17 2% of the dose) was within normal limits   This would be expected if the radioiodine were released as iodide, since it is known that in this condition the thyroid has an increased avidity for iodide

The total radioiodine in the thyroid, assumed to have been accumulated as iodide, is shown in Table II at the sixth and twenty-fourth hours

Radioactivity in the urine   In all cases, radioiodine was present in the earliest urine samples obtained, but the rate of excretion was much lower than the rate observed with comparable blood levels of radioiodide   In all the subjects the rate of excretion fell steadily from the second to the twenty-fourth hour (Fig 4)   This slow rate of fall contrasts with the rapid fall in the excretion rate which follows a single injection of radioiodide, and suggests a continuous liberation of iodide from the thyroxine   The initial high rate during the first hour is probably due to iodide present in the dose as injected

Although the greater part of the radioiodine in the urine is due to iodide, a small fraction has been found to be due to thyroxine   The I^{131} in urine samples from five subjects was analysed by butyl fractionation   The total recovery averaged 92% of the total in the urine   The proportion of the radioiodine in the butyl extract due to thyroxine averaged 9% of the total recovered, the values in 16 urine samples ranging from 5 7 to 23 8%   The remainder of the part recovered, appearing in the alkaline extract, is assumed to be iodide   In estimating the quantity of radioiodide and

TABLE II

Total radioiodine appearing as iodide at 6 and 24 hours calculated from the amount in the thyroid and 91% of the amount in the urine

Subjects 1 6   *dose given intravenously*
Subjects 7 and 8   *dose given by mouth*

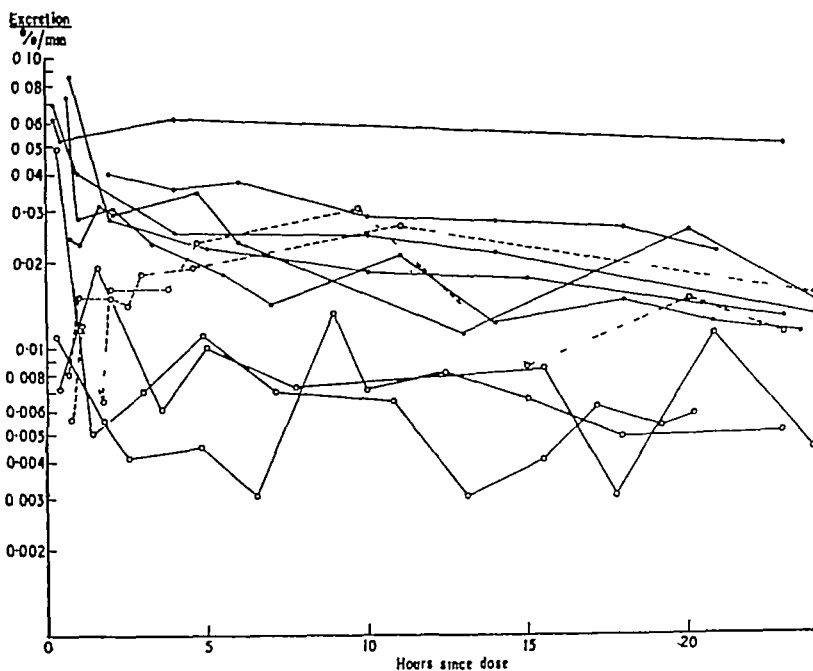| Subject	6 hours			24 hours		
	% in thyroid	% in urine	Total as iodide	% in thyroid	% in urine	Total as iodide
1	< 1	6 0	5 5+	2 0	20 3	20 5
2	5 2	13 0	17 1	10 1	21 3	29 5
3	4 0	11 1	14 1	11 0	27 2	35 8
4*	4 1	12 4	15 4	12 0	28 2	37 6
5*	4 5	9 4	13 0	12 5	23 5	33 0
6	< 1	7 3	6 7+	5 0	22 7	25 7
Average			11 9+			30 5
7	2 8	5 4	7 7	7 7	19 2	25 2
8	1 1	6 2	6 8	8 6	15 1	22 4

* *l* thyroxine used

Fig 4   Rate of urinary excretion of total radioiodine   After injection of sodium radiothyroxine, continuous line , after injection of naturally synthesised radiothyroxine, continuous line with circles , after oral sodium thyroxine, interrupted line

thyroxine in the urine, we have assumed in each case that 9% of the total radioiodine was thyroxine and that the remaining 91% was iodide   These are rough approximations based on the average values for all the samples   It is assumed that the variations observed were randomly scattered about a common mean and that the partition between iodide and thyroxine in the unrecovered radioiodine is the same as that in the butyl alcohol extract   This average value of 91% has been used to calculate the total radioiodide excreted in the urine up to the end of the 6th and 24th hours (Table II)

The renal clearance rate of radiothyroxine cannot be stated accurately because of the error in estimating absolute values for plasma and urinary radiothyroxine   But if we assume the figure mentioned above for urinary radiothyroxine, and that 90% of the plasma radioactivity was due to thyroxine, then the renal clearance rate for the injected thyroxine averages about 0 1 ml /minute

Release of radioiodide   It has not been possible to calculate the total radioiodide released from the thyroxine at these time intervals, because of the error in the estimate of plasma radioiodide and the difficulty in correcting for the time between release and excretion of iodide   But the sum of the totals in the thyroid and urine gives a minimum value (Table II)   It will be seen that, on the average, about a third of the labelling iodine is released by 24 hours

Entry into other tissues   (1) Red cells   The radioactivity in the red cells was measured in 21 blood samples from 4 cases   The mean ratio of the concentration in the cells to that in the plasma was 0 25 $\pm$ 0 04   There was no correlation between the ratio and the time interval after the injection   This suggests that equilibrium between plasma and red cells is established rapidly

(2) Fæces   Fæces were collected in 3 cases up to the third or fourth days   The total radioiodine at the end of the 3rd day is shown in Fig 5   The radioiodine in the fæces was not analysed chemically

(3) Concentration in the upper abdomen   In three cases, one or more days after the dose, measurements were made with the external counter placed successively in different positions at intervals of 5 cm down the anterior midline of the trunk   The counting rate decreased as the counter was moved below the thyroid and then rose again, reaching a peak value at the level of the xiphisternum (Fig 6)   In two cases, in whom measurements were made below this level, there was a second fall in the counting rate as the counter was moved below the umbilicus   The rise in counting rate at the xiphisternum cannot be explained by the relatively great depth of solid tissue which the counter ' sees " at this level, since it does not usually occur after a dose of radioiodide at a time when the plasma radioiodide concentration is still high (11)   Previous reports that thyroxine is concentrated selectively by the liver in animals (1, 4, 6) suggest that this may be

the site of accumulation of radiothyroxine in these cases   The counting
rate over this region cannot be expressed in terms of the proportion of the
dose

   The radiothyroxine space   The conventional " space " of a solute is
derived by dividing the total amount in the body by the plasma concen-
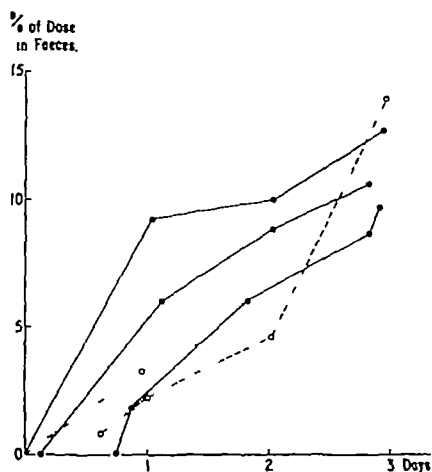tration   It cannot be assumed that the space calculated in this way for

Fig 5   Excretion of radioiodine in the fæces after intravenous (continuous line) and oral
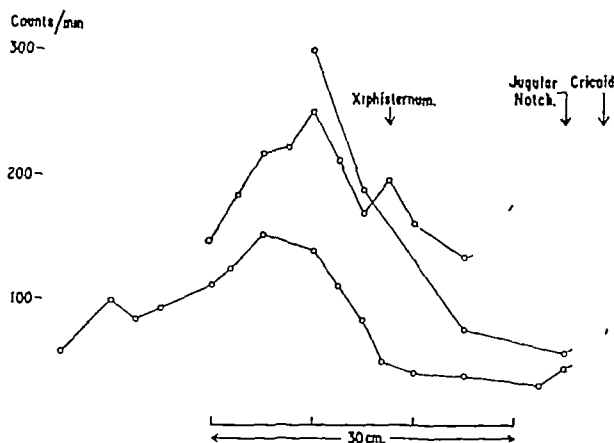(interrupted line) sodium radiothyroxine

Fig 6   External counting rate at successive positions down the anterior midline after intra
venous injection of sodium radiothyroxine

radiothyroxine corresponds to an actual volume of body fluid in which
thyroxine is evenly diffused, since we have not measured the concentration
in any extravascular fluid   Rather, there is evidence that a small
proportion of the dose is concentrated in the upper abdomen

However, the figure obtained for the radiothyroxine space, regarded simply as a ratio, gives a convenient expression for the distribution of a dose between the blood and the extravascular tissues at any moment

The amount of radiothyroxine left in the body at any time can be calculated by subtracting from the dose given, the sum of the total excreted
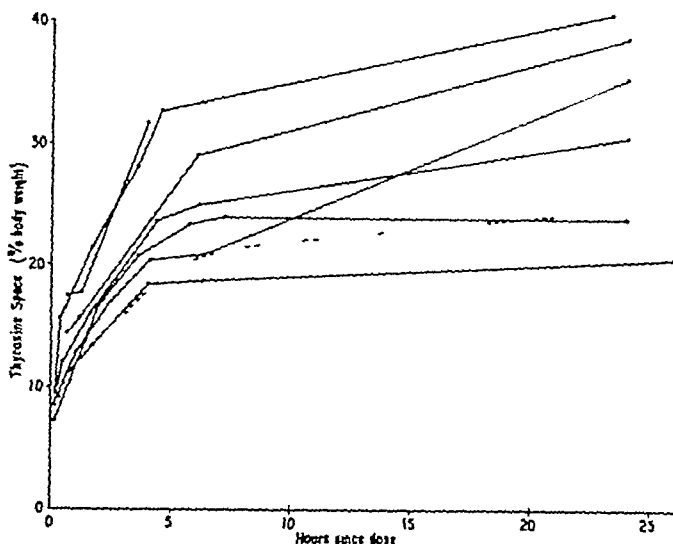

Fig 7   Continuous line, whole body space of radiothyroxine expressed as percentage of body weight, interrupted line, average values for proportional thigh space in the same subjects

TABLE III

Comparison of the distribution of radiothyroxine in the whole body and the thigh, at 1 6 and 24 hours after intravenous injection of sodium radiothyroxine

Subject	1 hour		6 hours		24 hours	
	Body space	Thigh space	Body space	Thigh space	Body space	Thigh space
1	12 2	7 5	20 6	14 5	35 4	15 0
2	15-0	12 0	29 0	25 0	38 4	20 5
3	13 7	8-0	24 7	19 5	30 6	27 0
4*	11 7	10 5	18 7	20 5	20 7	23 5
5*	12 2	8 5	23 5	19 5	35 4	22 5
6	13 2	13 0	20 7	24 0	35 4	40 0
Average	13 0	9 9	22 8	20 5	32 6	24 7

Body space expressed as a percentage of the body weight
Thigh space, proportion of a standard volume of thigh in which radioiodine is distributed (%)
* l thyroxine used

and the total metabolised   If it is assumed that this sum is equal to the total radioiodine accounted for in the urine and thyroid, and that the whole of the plasma radioiodine is due to thyroxine, then the values for the radiothyroxine space (Fig 7) may be calculated from the data presented above   In general, the space increases rapidly during the first 6 hours to an average value of 23% of the body weight   Then there is a further slow rise to an average of 33% at 24 hours (Table III)

In calculating the values for the radiothyroxine space, we have made certain assumptions

(1) The figure for the total radiothyroxine left in the body has not been corrected for loss in the fæces, since we do not know the time interval between excretion into the gut and appearance in the fæces However, if we assume an interval of 3 days, the values for the space at 6 hours would be reduced by 4 1, 3 8 and 3 7% of the body weight in the 3 cases in whom fæcal excretion was measured   If a shorter time interval is assumed, then the error would be smaller

(2) We have supposed that when the thyroxine molecule is broken down it releases its labelling iodine in a form which appears either in the thyroid or the urine   We have no direct evidence that this is true, and it is possible that a different type of breakdown occurs   For example, if the part A of the molecule (Fig 1) were released and remained in the body, but did not enter the thyroid, the total radiothyroxine in the body would be over-estimated by an amount equivalent to the fraction of the dose metabolised in this way   However, it seems unlikely that a breakdown of this sort gives rise to a large error, since significant amounts of radioiodine other than iodide and thyroxine are not detectible in the plasma (16)

(3) The nature of the radioiodine in the plasma has already been discussed, and reasons have been given for supposing that about 90% is due to thyroxine and that the remainder is iodide   As these figures are approximate, it seemed preferable to calculate the thyroxine space on the basis of the total plasma radioactivity and to state the error involved   The space calculated from total plasma radioiodine (V) will be intermediate between that for iodide (V_I) and that for thyroxine (V_T)   It can be shown that if a proportion p of the total radioiodine is present as iodide

$$V_T = \frac{V - V_I p}{(1 - p)} = \frac{V - 2 \cdot 5}{0 \cdot 9} \text{ litres}$$

taking p as 0 10 and V_I as 25 litres (10)   The average value of 17 litres, calculated for 1 hour, therefore exceeds the true thyroxine space by 0 9 litres

Distribution of radiothyroxine in the extravascular tissue   It has been shown that by the sixth hour, the radiothyroxine space is probably approaching a maximum value   At this time, about 87% of the dose is still in the body, and since the plasma concentration is then only about 5% of the dose per litre, we may assume that at least 70% of the dose is outside the bloodstream

We have no direct evidence on the distribution of this extravascular portion of the dose   However, estimates of the radioiodine space in the thigh made by a method previously described (10) show that the proportion of the thigh occupied by radioiodine is comparable with the whole body space for radiothyroxine expressed as a fraction of the body weight
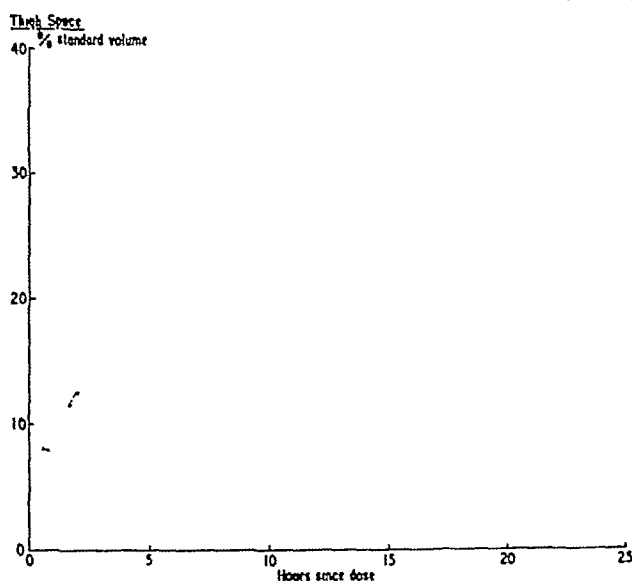

Fig 8   Proportion of a standard volume of thigh in which radioiodine is distributed after intravenous injection of sodium radiothyroxine

The values for the proportional thigh space for radioiodine are shown in Fig 8   It will be seen that the thigh space increases during the same period as the whole body space (expressed as a percentage of the body weight) and tends towards approximately the same maximum value at 6 hours   This correspondence is shown more clearly by comparing the average value for the proportional thigh space with the values for the whole body space (Fig 7)   In Table III the values for the 2 spaces are shown for each individual at 1, 6 and 24 hours   It can be seen that the proportional thigh space is usually a little lower than the whole body space

Results of oral administration

A dose of 20 microcuries of *dl*-thyroxine, with 80 micrograms of carrier, was given by mouth to each of 2 euthyroid subjects

Plasma concentration   In both cases the plasma concentration increased for the first 2 or 3 hours to a value of about 5% of the dose per litre, and then fell to about half this level by the 24th hour (Fig 2)   This gradual rise to a maximum differs markedly from the rapid rise seen after an oral dose of radioiodide

The I^{131} in 3 plasma samples from case 7 was analysed for organic and inorganic iodine   The proportion of the total radioactivity recovered in the butyl alcohol-averaged 81 5%, and the proportion of this faction due to thyroxine averaged 70 1%   None of the plasma samples from case 8 was analysed chemically

Accumulation in the thyroid, urine and faeces   A slow and roughly linear uptake was observed in the 2 cases   The time course of accumulation and the values at 6 and 24 hours (Table II) did not differ appreciably from the uptake following intravenous injection of the dose   For the reasons discussed above, it may be assumed, therefore, that the radioiodine entered the thyroid as iodide

The rate of urinary excretion followed a similar pattern in the 2 cases, increasing gradually to a maximum value of about 0 02% of the dose per minute and then falling continuously until the end of the 24 hour period (Fig 4)   The total radioactivity in the urine at the 6th and 24th hours (Table II) and the proportion of this total radioactivity recovered as thyroxine were comparable with the values observed after intravenous injection

Radioiodine in the faeces was measured in subject 7 up to the 3rd day and in subject 8 up to the 24th hour (Fig 5)   The data are insufficient for detailed comparison of the amounts excreted in the faeces after the oral and intravenous routes, but it appears unlikely that there is a marked difference between them

Results with radiothyroxine obtained by biosynthesis

Since a solution of sodium thyroxine may not behave identically as the circulating thyroid hormone, it seemed desirable to compare the fate of sodium thyroxine with that of a radioactive preparation of the natural hormone

Plasma for injection into normal subjects was taken from 3 thyrotoxic patients who had been given a therapeutic dose of radioiodide 3 days previously   Williams and others have shown that at this interval after a *tracer* dose of I^{131} more than 90% of the plasma radioactivity in thyrotoxic subjects is protein-bound (20), and we have confirmed this observation (11) The doses used in the treatment of the donor subjects did not exceed 10 mC but might still have caused the liberation of significant amounts of thyroglobulin by necrosis of thyroid cells   In 2 of the plasma samples, therefore, we attempted to test for the presence of radioactive thyroglobulin

by measuring the proportion of the total radioiodine which was removed by a single extraction with 5 volumes of butyl alcohol This proportion was compared with the proportion extracted from the plasma of thyrotoxic subjects given tracer doses of I^{131}, at the same interval after the dose The values after tracer doses averaged 72 3 $\pm$ 4 3% This did not differ significantly from the values of 67 4 and 72 5% obtained in the blood of the patients given therapeutic doses Since thyroglobulin is not soluble in butyl alcohol, this result indicates that thyroglobulin did not constitute more than a small fraction of the radioiodine in the plasma after the therapeutic doses

50 ml samples of blood were taken, with precautions for sterility, from each of 3 donors, and plasma obtained by centrifugation in heparinised McCartney bottles 20 ml samples were then injected into 3 euthyroid subjects, a small portion being kept for comparison with the radioactivity in the plasma and urine of these subjects In the first experiment, the plasma used for injection was kept overnight in the refrigerator, but in the other 2 the injection was made within $3\frac{1}{2}$ hours of drawing the blood

In each experiment, about 2 microcuries of radioiodine were injected Owing to the small size of the dose, data could be obtained only by liquid counting methods and chemical analysis of the radioiodine in the recipients could not be carried out

Plasma concentration The plasma levels differed strikingly from the values observed after intravenous injection of labelled sodium thyroxine (Table IV) In one case there was no significant change in the plasma concentration between 2 and 25 hours, and in the other 2 the fall was slight compared with that seen in the plasma concentration of sodium radiothyroxine Thus, the average concentration of natural labelled thyroxine at 24 hours was 14 8% of the dose per litre compared with 3 6% per litre for sodium thyroxine

The radiothyroxine space in these subjects could not be estimated, since the amount of radioiodine in the thyroid was too small to be measured externally However, a maximum estimate for the size of the space may be derived by dividing the total amount given less the fraction excreted in the urine, by the plasma concentration (Table V) Any correction for the fraction of the dose accumulated in the thyroid would reduce these figures

It is clear that natural thyroxine leaves the circulation more slowly than sodium thyroxine, as given in the experiments described here (Table IV)

Excretion in the urine Both the initial excretion rate of radioiodine and the total excreted by 24 hours were lower than the corresponding values for labelled sodium thyroxine (Figs 4 and 9) This difference in the amount

TABLE IV

Plasma concentration after intravenous injection of naturally synthesised radiothyroxine in 3 subjects

Subject	Time	%/l	Time	%/l	Time	%/l
a	2 hr 10′	16 0 ± 2 1	4 hr 30′	17 1 ± 2 0	25 hr	16 3 ± 3 2
b	2 hr 10′	25 2 ± 2 2			23 hr	18 5 ± 0 75
c			4 hr 45′	16 5 ± 1 8	24 hr	9 5 ± 2 3

Errors given are standard errors of counting

TABLE V

Maximum estimate for size of whole body thyroxine space after injection of naturally synthesised radiothyroxine

Subject	Time (hours)	Thyroxine space*
a	25	6 8
b	23	6 7
c	24	11 7
Average		8 4

* Thyroxine space as given by $\dfrac{100 - \% \text{ in urine}}{\text{plasma concentration}}$ and expressed as a percentage of body weight
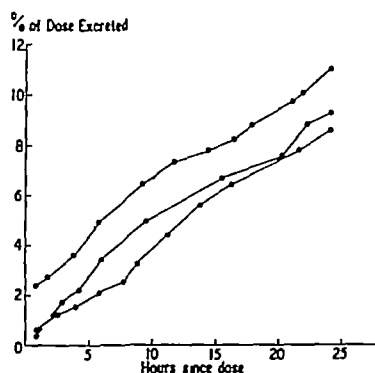

Fig 9   Excretion of radioiodine in urine after injection of naturally synthesised radiothyroxine

excreted is even more marked when the rate is related to the plasma concentration   Thus, the renal clearance of total radioiodine averaged 0 70, 0 27, and 0 36 ml /min in these 3 experiments, compared with the average value of 4 3 ml /min for the 6 subjects given labelled sodium thyroxine

The low rate of excretion of I^{131} in these cases suggests that natural thyroxine is more stable in the body than sodium thyroxine and therefore liberates inorganic iodine more slowly

Conjugation of thyroxine to protein It is known that the circulating thyroid hormone is bound to the plasma proteins, and that sodium thyroxine becomes protein-bound when added to plasma in vitro (19) The differences observed between natural and synthetic radioactive thyroxine could be explained if injected sodium thyroxine remained freely diffusible for several hours before being conjugated to the plasma proteins We tested this possibility by measuring the rate at which radiothyroxine becomes precipitable by trichloracetic acid after addition to human plasma in vitro

Contaminating radioiodide was removed from the radiothyroxine by filter paper chromatography The sample was allowed to run on the paper strip for 12 hours The positions of the iodide and thyroxine fractions were then found from an X-Ray film developed after contact with the strip The section of the strip containing the thyroxine was washed with M/50 Na_2CO_3 This solution contained about 10 uC of radiothyroxine in 1 c c and 0 1 c c was added to each of 4 centrifuge tubes containing 2 c c of plasma Two ml of 20% trichloracetic acid were then added at 1 minute, 15 minutes, 2 hours and 4 hours after the addition of radiothyroxine The supernatant solution was removed after centrifugation and the protein precipitate washed once with 5 c c of M/50 Na_2CO_3 The protein precipitate was dissolved in N/10 NaOH Total radioiodine was then measured in the combined supernatant solution and washing, and in the protein solution A similar series of measurements was made, using radioiodide instead of radiothyroxine

TABLE VI

Time after addition of radiothyroxine	% protein bound I^{131} of total recovered	% protein bound I^{131} after addition of radioiodide
1 min	98 7	3 4
15 min	98 4	6 2
2 hr	97 2	11 4
4 hr	98 6	4 3

The results (Table VI) show that in the conditions of this experiment radiothyroxine is rapidly and completely bound to protein and therefore give no support for the hypothesis that injected sodium thyroxine remains diffusible in vivo for several hours

DISCUSSION

The sodium thyroxine space The similarity between the entry of radioiodine into the thigh and the whole body indicates that radiothyroxine approaches an equilibrium in which the greater part is evenly distributed throughout the body The figures obtained during the first 6 hours are consistent with diffusion into the extracellular fluid since the values at the end of this period (after correction for excretion in the fæces assumed to equal 10% of the dose) are about equal in range and average to the values reported for the inulin space (15) The continued slow increase in the size of the space may represent entry into the extracellular fluids However, in the absence of further data both these suggestions can be little more than speculation, and it remains possible that the increase in the size of the space is due to selective concentration in localised regions

Our observation that injected sodium thyroxine leaves the bloodstream rapidly may appear to conflict with current opinions about the nature of the circulating hormone It has recently been shown (7, 16) that the thyroid hormone circulates as thyroxine, and not as a thyroxine peptide But it is known that the hormonal iodine in the plasma cannot be removed by dialysis and that thyroxine added to plasma becomes loosely conjugated to protein (19) Salter has also shown that the concentration of iodine is much lower in the extravascular fluids than in the plasma, and has suggested that thyroid hormone leaves the bloodstream by slow leakage of albumen into the extracellular fluid (12) Our results with natural radiothyroxine also indicate that the circulating hormone leaves the bloodstream more slowly than injected sodium thyroxine We cannot explain these discrepancies, but several possibilities may be mentioned

The linkage between sodium thyroxine and the plasma proteins may differ from the linkage present in the natural hormone We have seen that the conjugation of sodium thyroxine to protein is very rapid, but the resulting protein-thyroxine complex may be less stable in vivo than the circulating hormone formed by natural synthesis Salter and Johnston (14) have shown that thyroxine added to plasma differs in certain respects from the organic iodine normally circulating in the blood Thus, thyroxine added to the plasma of myxœdematous patients is not precipitated by all those reagents which precipitate the plasma hormonal iodine It seems clear, therefore, that we cannot assume that the thyroid hormone and injected thyroxine circulate in the same physical state

Differences in the amount of carrier thyroxine introduced with the dose may account for a difference between the rates of disposal of the two types of radiothyroxine The thyroxine content of each 20 c c sample of plasma containing natural radiothyroxine would not appreciably change the total quantity of circulating thyroxine But the 80 micrograms given with each dose of sodium radiothyroxine might increase the normal blood

concentration of organic iodine if distributed through a blood volume of 5 litres Evidence mentioned later indicates that large doses of thyroxine are removed from the bloodstream by mechanisms which may not operate in natural conditions It is possible that in the experiments with sodium thyroxine a critical threshold is exceeded so that part of the dose is removed by a path not followed in physiological conditions

Differences in the position of the labelling iodine atoms might cause an apparent difference in the fate of the two types of thyroxine In the samples of sodium thyroxine, each radioactive molecule was labelled by 2 radioactive iodine atoms in the 3' and 5' positions But in the samples of naturally synthesised thyroxine, the radioactive hormone must have consisted largely of a mixture of molecules labelled by a single radioiodine atom in each of the 4 possible positions The reason for this is as follows the number of I^{131} atoms in the therapeutic dose is so small that 10 millicuries contribute only about 1 in every 10^9 of the iodine atoms circulating during the initial phase of accumulation in the thyroid The chance that any given precursor molecule of thyroxine will be iodinated by a radioactive atom is therefore about 1 in 10^5 and the chance that the same molecule will receive 2 or more radioactive atoms is negligible Moreover, since the gland must, at any moment, contain precursor molecules which have no iodine atoms and some which already have 1, 2 and 3 atoms of I^{127}, iodination by radioiodine must take place at each of the 4 positions If the iodine at 3' and 5' positions were more rapidly detached from the molecule than at 3 and 5 positions, then the radioiodine concentration in the plasma would fall more rapidly after an injection of the sodium thyroxine used in these experiments

The two types of thyroxine also differed in that all except 2 of the samples of sodium thyroxine were racemic mixtures whereas the biosynthesised hormone contained only levo rotatory thyroxine However, it appears unlikely that this can account for the differences observed between sodium thyroxine and natural thyroxine, since the values for the *l* and *dl* samples of sodium thyroxine were roughly similar

Concentration in the upper abdomen Early work with non-radioactive thyroid hormone has shown that when large doses of *dl*-thyroxine are given by mouth to animals, a large proportion of the dose is re-excreted by the liver (1) Gross and Leblond (4) have demonstrated a similar excretion by the liver when radioactive thyroxine, labelled with large carrier doses, is injected intravenously into rats With doses containing 1 mg of thyroxine, as much as 80% of the iodine may appear in the fæces by the 24th hour However, later work with small doses of labelled thyroxine suggests that the high concentrations reported in the liver after large doses are due to an overflow mechanism which predominates when the blood level of thyroxine rises much above normal (8)

The high counting rate found over the upper abdomen in the subjects injected with radiothyroxine is consistent with a similar concentration in the liver in man We have no method of calibrating the external counting

rate in this region in terms of the proportion of the dose present, but the small quantities of radioiodine recovered in the fæces suggest that only a small proportion of a tracer dose leaves the circulation by excretion in the liver   It appears unlikely, therefore, that this mechanism is of much importance in the metabolism of circulating thyroxine in physiological conditions in man

The turnover time of thyroxine   Several workers have shown that exogenous thyroxine is broken down in the peripheral tissues of animals, with the liberation of inorganic iodine (1, 3, 21)   Taurog, Chaikoff and Entenman (17) attempted to measure the turnover time of thyroxine in the extrathyroid tissues of the dog, from the rate of fall of plasma radio-thyroxine concentration after a single injection of radioactive thyroid hormone removed from the blood of rats   They concluded that the whole of the circulating thyroxine was renewed from the thyroid about once every 6 hours   However, the disappearance rate of radiothyroxine in the plasma is not a valid measure of the overall turnover time in the periphery, and may be determined chiefly by the speed with which equilibrium is established between intravascular and extravascular thyroxine   A more satisfactory measure of the rate at which thyroxine is metabolised could be obtained from the rate at which the labelling iodine is liberated

Our measurements on the total radioiodine appearing in the thyroid and urine indicate that the rate of turnover of sodium thyroxine in man is slower than that estimated by Chaikoff *et al* for the dog   The experiments with sodium thyroxine suggest that about a third of the labelling iodine is released by 24 hours and the experiments with natural thyroxine point to an even slower rate of breakdown in physiological conditions

The existence of an iodine cycle normally occurring in man has been postulated on theoretical grounds (13) and it has been shown that when large doses of thyroxine are given to human subjects by injection, iodide appears in the urine (3)   Our results with tracer doses of radioactive thyroxine show that this type of breakdown also occurs in physiological conditions and must therefore be part of the normal metabolic cycle of iodine   It is evident, also, that the iodine is liberated in tissues where it becomes rapidly available for re-accumulation in the thyroid and resynthesis to thyroxine

Absorption from the gut   Widely discrepant results have been reported for the efficiency of absorption from an oral dose of thyroxine in animals, the figures varying from 1/16 (2) to $\frac{1}{2}$ (9)   These discrepancies may in part be due to differences in the quantity of material used, but it is also known that absorption varies with the solubility of the thyroxine preparation   Thus, Thompson and co-workers have shown that disodium thyroxine is 100 times, and monosodium thyroxine 30 times, as effective as crystalline thyroxine when given by mouth for the treatment of myxœdema (18)

The rate of absorption of a tracer dose of monosodium radiothyroxine cannot be estimated from the plasma concentration ⁻curves in our experiments, because the quantity of radioiodine in the blood at any moment is determined by the amount which has entered from the gut and the turnover time in the circulation However, the continued high plasma concentration of radioiodine after an oral dose shows that absorption is slow and continues for several hours, since we have shown that after a single injection, radiothyroxine rapidly disappears from the bloodstream

It is impossible, with the few data available, to give more than a very rough estimate of the proportion of the dose finally absorbed The similarity between the figures for fæcal excretion in subject 8 and in the 6 subjects given the dose by injection is consistent with complete absorption, but we cannot distinguish between incomplete absorption and total absorption with partial re-excretion Some of the absorption of radioiodine may occur as iodide released by digestion of thyroxine in the gut, but the high proportion of organic iodine in the plasma radioiodine and the low total excretion of iodide in the urine following the oral dose show that this can occur only to a small extent

Summary

1 When radioactive sodium thyroxine is given intravenously to normal humans, radioiodide is continuously released and appears in the urine and thyroid By 24 hours an average of 30% of the labelling iodine accumulates in these two sites

2 Most of the radioiodine in the urine is due to iodide, but about 10% is due to thyroxine

3 About 10% of the radioiodine in the dose appears in the fæces by 3 days

4 The external counting rate over the upper abdomen suggests that some radioiodine is concentrated in the liver

5 The sodium thyroxine space rises rapidly to a value of 23% of the body weight by 6 hours, and then increases more slowly for the following 18 hours The sodium thyroxine space in a standard volume of thigh is comparable in extent and rate of change with the whole body space

6 After oral sodium radiothyroxine, the accumulation of radioiodine in the thyroid, urine, and fæces, and the composition of the plasma radioiodine are comparable with the values obtained after injection It seems, therefore, that after an oral dose most of the thyroxine is absorbed as such from the gut

7 Biosynthesised radiothyroxine was obtained from the blood of patients given large doses of radioiodide The fate of this material, when injected, suggests that the natural circulating hormone is more stable in the body and is less diffusible than sodium thyroxine

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